2007

Physical Characteristics Of An Individual: The Identification Of Biomarkers For Biological Age Determination

Michelle Alvarez
University of Central Florida

PHYSICAL CHARACTERISTICS OF AN INDIVIDUAL: THE IDENTIFICATION OF BIOMARKERS FOR BIOLOGICAL AGE DETERMINATION

by

MICHELLE STEFANIW-ALVAREZ
B.S. University of Central Florida, 2002

A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Biomedical Science
in the Department of Chemistry
in the College of Sciences
and the Burnett College of Biomedical Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2007

Major Professor: Jack Ballantyne

ABSTRACT

It is now a matter of routine for the forensic scientist to obtain the genetic profile of an individual from DNA recovered from a biological stain deposited at a crime scene. Potential contributors of the stain must either be known to investigators (i.e. a developed suspect) or the questioned profile must be searched against a database of DNA profiles such as those maintained in the CODIS National DNA database. However, in those instances where there is no developed suspect and no match is obtained after interrogation of appropriate DNA databases, the DNA profile *per se* presently provides no meaningful information to investigators, with the notable exception of gender determination. In these situations it would be advantageous to the investigation, if additional probative information could be obtained from the biological stain. A useful biometric that could provide important probative information, and one that may be amenable to molecular genetic analysis, is the biological age of an individual. The ability to provide investigators with information as to whether a DNA donor is a newborn, infant, toddler, child, adolescent, adult, middle-aged or elderly individual could be useful in certain cases, particularly those involving young children such as kidnappings or in providing additional intelligence during terrorist investigations. Currently no validated molecular assays exist for age determination.

Biological human ageing can be defined by two distinct processes, degenerative and developmental ageing. The degenerative process of ageing is based on theories which

identify an increase or decrease in physiological conditions with increasing age. In contrast, the developmental process of ageing is based on the theory that as individuals increase in chronological age, there will be subtle corresponding molecular based biological changes, each requiring genes to be expressed or silenced, indicative of that particular stage of life.

We investigated the degenerative process of chromosomal telomere shortening, as well as the developmental process of gene expression profiling analysis, in an attempt to identify biomarkers of biological age in a self-renewing tissue such as blood. While telomere length analysis was an ineffective method for age determination; gene expression analysis revealed three gene transcripts expressed in an age-dependent physiological manner. These species namely- COL1A2, HBE1 and IGFBP3, were found to be expressed at elevated levels in younger individuals, newborns, or post-pubertal individuals, respectively.

The biological process of hemoglobin switching was also investigated for the possibility of determining human age. While experimenting with the potential of using the gamma-hemoglobin chains, as newborn specific gene candidates, we serendipitously discovered four novel truncated transcripts, which we have termed HBG1n1, HBG1n2, HBG2n2 and HBG2n3; whose expression was restricted to whole-blood newborn samples and specific fetal tissues. The molecular origin of these transcripts appears to be at the RNA level, being produced by specific rearrangement events occurring in the standard gamma hemoglobin transcripts (HBG1 and HBG2), which yield these new isoforms that are expressed in a highly regulated tissue specific manner.

To my love, Carlos Humberto and my life, Vincent Charles

ACKNOWLEDGMENTS

To my advisor, Dr. Jack Ballantyne, thank you for your guidance and for sharing your knowledge and experience in molecular genetics and forensic science. It has been an honor and a pleasure to work with such a brilliant person and talented scientist all these years. I will always be grateful for the amazing opportunities that I had while working in your lab.

I would like to thank the members of my committee, Drs. Chris Parkinson, Annette Khaled, and Saleh Naser, for their time and helpful input during the course of this project.

I also want to express my gratitude to all of my colleagues in the Ballantyne Lab, especially Dr. Jane Juusola, thanks for the great collaboration; I could not have succeeded without you.

Finally, thank you to my family, Carlos and Vincent, for your continuous support and encouragement of my dreams and for accompanying my on this journey. To my parents and brother, thank you for your support.

This project was partially funded by the Technical Support Working Group (TSWG) on Counterterrorism (Contract # DAAD05-03-C-0047; TSWG Task IS-FO-1594), by the National Institute of Justice (Contract # 2005-MU-BX-K075), and by the state of Florida through the University of Central Florida's National Center for Forensic Science.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS/ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
CODIS	combined DNA index system
cDNA	complementary DNA
cRNA	complementary RNA
Ct	cycle threshold
dCt	delta cycle threshold
DNA	deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
6-FAM	6-carboxyfluorescein
GNAS	guanine nucleotide binding protein, alpha stimulating
GOI	gene of interest
HBG1	hemoglobin gamma chain 1
HBG1n1	hemoglobin gamma chain 1 newborn 1
HBG1n2	hemoglobin gamma chain 1 newborn 2
HBG2	hemoglobin gamma chain 2
HBG2n2	hemoglobin gamma chain 2 newborn 2
HBG2n3	hemoglobin gamma chain 2 newborn 3
IPC	internal positive control
IVT	*in vitro* transcription
MGB	minor groove binding

mRNA	messenger RNA
NCBI	National Center for Biotechnology Information
NFQ	non-fluorescent quencher
NTC	non-template control
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
qRT-PCR	quantitative reverse transcription-polymerase chain reaction
RNA	ribonucleic acid
ROS	reactive oxygen species
RT	reverse transcription
RT-PCR	reverse transcription-polymerase chain reaction
S15	ribosomal protein S15 subunit
SWGDAM	scientific working group on DNA analysis methods

CHAPTER ONE: INTRODUCTION

It is now a matter of routine for the forensic scientist to obtain the genetic profile of an individual from DNA recovered from a biological stain deposited at a crime scene. Potential contributors of the stain must either be known to investigators (i.e. a developed suspect) or the questioned profile must be searched against a database of DNA profiles such as those maintained in the CODIS National DNA database [1]. However, in those instances where there is no developed suspect and no match is obtained after interrogation of appropriate DNA databases, the DNA profile *per se* presently provides no meaningful information to investigators, with the notable exception of gender determination [2]. In these situations it would be advantageous to the investigation, if additional probative information could be obtained from the biological stain. Additional investigative parameters could include determining the physical characteristics of the individual depositing the biological stain. A number of physically recognizable characteristics of an individual are at least partly inherited and these include skin-, hair- and eye- color, stature (height and weight) and facial morphology [3-7]. Theoretically, and given sufficient knowledge of the genetics of complex polygenic traits, DNA analysis on a crime scene sample could provide investigators with information akin to eyewitness identification. Since, with few exceptions, our understanding of the genetics of these complex traits is somewhat rudimentary, development of significant forensic applications awaits further advances in our knowledge in this area. One exception may be skin and hair pigmentation since to a large degree the genetics of pigmentation has

proved to be amenable to molecular genetic analysis [3, 4, 8]. An additional useful biometric that could provide important probative information, and one that may be amenable to molecular genetic analysis, is the biological age of an individual. The ability to provide investigators with information as to whether a DNA donor is a newborn, infant, toddler, child, adolescent, adult, middle-aged or elderly individual [9] could be useful in certain cases, particularly those involving young children such as kidnappings or in providing additional intelligence during terrorist investigations. Currently no validated molecular assays exist for age determination. Two approaches have been evaluated for their ability to identify biomarkers associated with biological age; messenger RNA profiling and telomere length analysis.

Biological human ageing can be defined by two distinct processes, degenerative and developmental ageing. The degenerative process of ageing is based on theories which identify an increase or decrease in physiological conditions with increasing age. Postulated molecular mechanisms of the degenerative ageing process include, inter alia, progressive damage to DNA, including mitochondrial DNA mutations, deletions, and insertions [10-13], the shortening of telomeric regions on the ends of chromosomes [14, 15], long-lived protein glycation [16], and reactive oxygen species (ROS)-mediated oxidative damage to macromolecules [17-19]. These degenerative studies attempt to correlate specific molecular damage with increases in age, particularly in post-reproductive individuals [20]. From a forensic standpoint however, and in contrast to the degenerative process of ageing, it would be useful to be able to distinguish between individuals of all age groups, but this may require the detection of more subtle molecular changes. Thus we have considered an alternative approach to age determination that is

based upon the epigenetic and developmental control of gene expression that occurs during all stages of human development [9].

The developmental process of ageing is based on the theory that as individuals increase in chronological age, there will be subtle corresponding molecular based biological changes, each requiring genes to be expressed or silenced indicative of that particular stage of life. Using this approach to biological age determination, every stage of the human lifecycle (birth through death) [9] can be defined by identifying sub-sets of the 20-25 thousand human genes [21] that will be differentially expressed in correlation with a specific developmental stage [22]. Theoretically, a comparison of the gene expression profile from individuals of different ages could reveal constellations of candidate genes whose expression is correlated with a specific age. A number of recent reports have described age-associated differential gene expression profiles in skeletal muscle [23, 24], liver [25], brain [26], teeth [27, 28] and skin [29, 30].

Determining the biological age of an individual depositing a body fluid at a crime scene was evaluated by differential gene expression analysis using the Affymetrix GeneChip® as well as PubMed literature searches of age related gene expression differences. Candidate genes were generated from the Affymetrix array by performing three two-group comparisons between newborn, juvenile and elderly male individuals. A clear example of developmental age related differential gene expression, is that of hemoglobin gene switching [31, 32]. The human β-hemoglobin locus is located on the short arm of chromosome 11 (11p15.5), and encodes five functional β-like globin genes, ε, $^{G}\gamma$, $^{A}\gamma$, δ, and β, and a non-functional β-pseudogene ($^{\beta}\psi$) [33, 34]. The expression of embryonic hemoglobin (ε-globin) commences in the yolk sac in the early stages of

gestational development, approximately during week two and continues until six weeks (37 days) postconception [35]. During the next six weeks of gestation (days 37-79), the newly developed fetal liver and fetal spleen begin to produce the fetal specific gamma globin chains ($^{A}\gamma$ and $^{G}\gamma$) of fetal hemoglobin [35]. This increased production of γ-globin is accompanied by a shutdown of ε-globin synthesis. Beginning at approximately 20 weeks gestation and continuing throughout life, adult β-globin gene expression commences in the bone marrow and γ-globin expression is down regulated [35]. This biological process of hemoglobin switching was investigated for the possibility that the detection of gamma (γ)-globin messenger RNA (mRNA) in a bloodstain would be indicative of a newborn baby. While experimenting with the potential of using the gamma-hemoglobin chains as newborn specific gene candidates, we serendipitously discovered four truncated mRNA transcripts, which we have termed HBG1n1, HBG1n2, HBG2n2 and HBG2n3. These novel isoforms exhibit a highly regulated expression pattern, only be formed in newborn blood, fetal liver and spleen, and to a lesser extent fetal heart, thymus and kidney. The molecular origin of these transcripts appears to be at the RNA level, being produced by specific rearrangement events occurring between direct repeat sequences located exons two and three of the standard gamma hemoglobin mRNA transcripts (HBG1 or HBG2).

CHAPTER TWO: RESEARCH DESIGN AND METHODOLOGY

Messenger RNA Profiling Analysis for Biological Age Determination

Affymetrix GeneChip® Whole Blood RNA Isolation and Sample Preparation

Human blood samples were obtained from donors from Florida Hospital (Orlando, FL) after receiving exemption from the Hospital's Institutional Review Board and in accordance with procedures approved by the University of Central Florida's Institutional Review Board (**APPENDIX H: UCF IRB FORM**). Whole blood samples from three male newborns (all <24-hours old), three male juveniles (14-, 15- and 18-years old) and three male elderly (74-, and 79- (x2), years old), were transferred from sterile EDTA collection tubes into PAXgene™ Blood RNA Tubes (QIAGEN Inc., PreAnalytiX, Valencia, CA), which concentrate and stabilize cellular RNA, protect degradation by RNases and prevent induction of gene expression [36-38]. PAXgene™ Blood RNA tubes were incubated at room temperature overnight, and processed by the manufactures instructions [36-38]. Extracted RNA was shipped overnight to Expression Analysis (Durham, NC) for hybridization to the GeneChip® Human Genome U133 Plus 2.0 Array (HG-U133+2), which analyzes the expression levels of over 47,000 transcripts and variants, including 38,500 well-characterized human genes derived from GenBank®, dbEST, and RefSeq [39].

Expression Analysis Processing of Total RNA for Affymetrix GeneChip® Hybridization

The ribosomal subunit 28S/18S peak ratio was assessed for quality control of the submitted total RNA samples via the Agilent 2100 Bioanalyzer [40]. For low quantity (10-100 ng) samples, total RNA undergoes to a two-cycle target labeling procedure, before GeneChip® hybridization [41]. Briefly, total RNA is submitted to a first cycle, first strand cDNA synthesis reaction with T7-Oligo(dT) primers, followed by a first cycle, second strand cDNA synthesis reaction to produce double-stranded T7-labeled cDNA. A first cycle *in vitro* transcription (IVT) reaction containing unlabeled ribonucleotides is performed; and the newly synthesized unlabeled cRNA is reverse transcribed, using random primers, in the second cycle, first strand cDNA synthesis step. In the second cycle, second strand cDNA synthesis reaction the T7-Oligo(dT) promoter primer is used to generate a double-stranded cDNA template containing T7 promoter sequences. A second IVT reaction is then performed with biotinylated ribonucleotides to label the antisense cRNA. The labeled cRNA is then cleaned, fragmented, and hybridized to the AFFYMETRIX GeneChip® expression array (**Figure 1**) [41].

Statistical Evaluation of Hybridization Intensities on the Affymetrix GeneChip®

GeneChip algorithms are a set of rules and calculations used to derive biologically meaningful results from hybridization intensities measured on GeneChip expression probe arrays. A particular transcript is represented by a probe set with 15-20 probe pairs, containing perfect match (PM) and mismatch (MM) probe cells, which are used to generate discrimination scores followed by detection *p*-values. The discrimination score

(R) is determined by the ability of a probe pair to detect its intended target, by measuring the target-specific intensity difference of the probe pair (PM-MM) relative to its overall hybridization intensity (PM+MM) (**Figure 2**) [42]. Discrimination score values are then compared to the threshold Tau (default value = 0.015) and the detection *p*-value is determined by One-sided Wilcoxon's Signed Rank test, which assigns each probe pair a value based on how far the R-score is from Tau [42]. Once detection *p*-values are calculated, the algorithm for comparison analysis is used [43] to determine the relative change in abundance for each transcript between a baseline and an experimental sample set. Comparison analysis generates two transcript expression estimates between different data arrays: change significance and change quantity metrics. Change significance values calculate an increase, decrease or no change in gene expression, while change quantity estimates the magnitude and direction of transcript change [42].

Blood Sample Preparation

Human blood samples were obtained from donors from Florida Hospital (Orlando, FL) after receiving exemption from the Hospital's Institutional Review Board and in accordance with procedures approved by the University of Central Florida's Institutional Review Board (**APPENDIX H: UCF IRB FORM**). Bloodstains were made by dispensing 50-μL aliquots onto sterile cotton gauze and air-dried overnight at room temperature.

RNA Isolation

A guanidine isothiocyanate-phenol:chloroform extraction method was used [44]. Briefly, 500-µL denaturing solution (4M guanidine isothiocyanate, 0.02M sodium citrate, 0.5% sarkosyl, 0.1M β-mercaptoethanol) was preheated in a Spin-Ease™ extraction tube (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD) at 56ºC for 10 minutes. Prepared stains were then added and incubated at 56ºC for 30 minutes. The stain was removed into a Spin-Ease™ extraction tube filter insert, placed back inside the extraction tube and centrifuged for 5 min at 16,000g, after which the filter and the fabric remnants were discarded. Fifty microliters of 2 M sodium acetate and 600-µL of acid phenol:chloroform 5:1, pH 4.5 (Ambion Inc., Austin, TX) were added to the extract, and incubated at 4ºC until two phases were resolved (~20 minutes), then centrifuged at 16,000g for 20 minutes. The RNA-containing aqueous phase was transferred to a sterile microcentrifuge tube, along with 30-µg GlycoBlue™ glycogen carrier (Ambion Inc., Austin, TX) and precipitated with 500-µL isopropanol overnight, at -20ºC. Samples were then centrifuged at 16,000g for 20 minutes to pellet the RNA. The supernatant was carefully removed and the pellet washed once with 1-mL 75% ethanol/25% DEPC-treated water and re-centrifuged at 16,000g for 10 minutes. The supernatant was discarded, the pellet dried in a vacuum centrifuge for 3-5 minutes and re-solubilized in 17-µL of RNAsecure Resuspension Solution (Ambion Inc., Austin, TX) at 60ºC for 10 minutes. RNA samples were treated with DNase I immediately or stored at -20ºC.

DNase I Digestion

Total RNA was treated with six units of TURBO™ DNase (RNase-Free) (2 U/µL) (Ambion Inc., Austin, TX) at 37ºC for 1-2 hours. The TURBO™ DNase was inactivated at 75ºC for 10 minutes, the samples chilled on ice and then stored at -20ºC until needed [45, 46].

Quantification of Nucleic Acids

RNA was quantified using a sensitive fluorescence assay based upon the binding of the unsymmetrical cyanine dye RiboGreen® (Molecular Probes, Eugene, OR) [47]. The manufacturer's instructions were followed for the high-range assay, which detects from 20-ng/mL to 1-µg/mL. Briefly, 200-µL assays comprised of 2-µL TURBO™ DNase treated RNA extract, 98-µL TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5, in nuclease-free water), and 100-µL 750 nM RiboGreen® reagent in a 96-well plate format. After RiboGreen® addition and a three minute incubation at room temperature protected from light, fluorescence emission at 535 nm (excited at 485 nm) was determined using a Wallac Victor2 microplate reader (Perkin Elmer Life Sciences, Boston, MA). RNA concentration was calculated using an appropriate standard curve as described by the manufacturer [47]. All RNA samples were diluted to 5ng/uL with nuclease-free water (Ambion Inc., Austin, TX).

Reverse Transcription (cDNA Synthesis)

For all samples 6-µL of RNA (30-ng), was heated at 75ºC for 3 minutes and combined with 4-µL of a 10 mM dNTP mix (Applied Biosystems, Foster City, CA), 2-µL of 10X first-strand buffer (500 mM Tris–HCl pH 8.3, 750 mM KCl, 30 mM $MgCl_2$, 50 mM DTT), 2-µL Random Decamer primers (50-µM), 20-units SUPERase-In™ RNase Inhibitor (20 U/µL) (Ambion Inc., Austin, TX), 100-units Moloney Murine Leukemia Virus-Reverse Transcriptase (100 U/µL) (Ambion Inc., Austin, TX) and nuclease-free water (Ambion Inc., Austin, TX) were added to yield a final reaction volume of 20-µL. Reaction mixtures were incubated at 42ºC for 1 hour and 95ºC for 10 minutes to inactivate the reverse transcriptase [48, 49].

Polymerase Chain Reaction

All single gene amplification reactions were conducted in a total volume of 25-µL. Three nanograms of cDNA was amplified with a standard reaction mix containing 1x PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.125 mM each dNTP, 0.4 µM primers (**APPENDIX E: CANDIDATE GENE PRIMER SEQUENCES FOR RT-PCR**) and 1.25-units AmpliTaq Gold™ DNA polymerase (5 U/µL) (Applied Biosystems, Foster City, CA). Nuclease-free water (Ambion Inc., Austin, TX) was added to yield the final reaction volume.

Standard PCR conditions consisted of an 11 minute denaturing step at 95ºC followed by 35 cycles at (1) 94ºC; 0:20 (2) 55ºC or 60ºC; 0:30 (3) 72ºC; 0:40 and a final extension step (72ºC; 10:00) [50, 51].

Post RT-PCR Electrophoresis

RT-PCR amplified products were visualized on 4% NuSieve® GTG® Agarose gels (Cambrex Bio Science Rockland, Inc., Rockland, ME). Electrophoresis was carried out at 100V for 1.25 hours in TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer. Gels were stained with SYBR® Gold nucleic acid stain (Molecular Probes, Eugene, OR), visualized on the Omega10 Chemiluminescence Imaging System (MLTRA-LUM, Inc., Claremont, CA) and analyzed with ONE-Dscan 2.05, 1-D Gel Analysis Software for Windows (Scanalytics, Inc., Fairfax, VA).

Real-Time PCR

All singleplex qRT-PCR assays were performed in a 25-µL total reaction volume consisting of a standard reaction mix containing: three nanograms of cDNA, 12.5-µL 2x Taqman® Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.40-µM each forward and reverse primer, 0.25-µM of each probe and nuclease-free water (Ambion Inc., Austin, TX). Primer and probe sequences are listed in **APPENDIX G: CANDIDATE GENE PRIMER SEQUENCES FOR qRT-PCR**.

Three optimized duplex real-time PCR reactions consisted of the following changes to the standard reaction mix: COL1A2 0.9-µM primers to S15 0.025-µM primers; HBE1 0.2-µM primers to S15 0.4-µM primers, and IGFBP3 1.2-µM primers to S15 0.05-µM primers.

Real-Time PCR reactions were carried out on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA). Amplification conditions consisted of: (1) 1 cycle

of 50˚C; 2:00 (2) 1 cycle of 95˚C; 10:00 (3) 50 cycles of 95˚C; 0:15 and 60˚C; 1:00. Data was collected at stage 3, step 2 (60˚C; 1:00). Delta cycle threshold (dCt) values were calculated by subtracting the Ct value generated from the age specific gene of interest (GOI) from the Ct value of the housekeeping gene (i.e. dCt = Ct (S15) – Ct (GOI)) [52]. Samples which fail to amplify the GOI are given a default Ct value of 40.000 or 50.00 (the amount of qPCR cycles used).

Fetal Specific Isoforms of Gamma Hemoglobin as Biomarkers for Biological Age Determination

Sample Preparation

Human blood samples were obtained from donors from Florida Hospital (Orlando, FL) after receiving exemption from the Hospital's Institutional Review Board and in accordance with procedures approved by the University of Central Florida's Institutional Review Board (**APPENDIX H: UCF IRB FORM**). Bloodstains were made by dispensing 50-µL aliquots onto sterile cotton gauze and air-dried overnight at room temperature. Other body fluid samples were collected from volunteers in accordance with guidelines approved by the UCFIRB. Saliva and semen samples were obtained from healthy individuals and 50-µL stains prepared. Buccal swabs, vaginal secretion swabs and menstrual blood swabs were obtained from healthy individuals and allowed to air-dry overnight at room temperature. Venous blood, saliva and vaginal secretion swabs obtained from an expectant mother at various time points throughout the pregnancy and

breast milk swabs (1-month post delivery) were air dried overnight. All stains were stored at -45ºC until needed.

For stability studies, venous blood (50-µL) was prepared on sterile cotton gauze and allowed to sit at room temperature (~25ºC) for one, three, six, nine, twelve and fifteen months. Animal blood (with biological age, if known) for species specificity testing was collected from two Pigtailed Macaques (22-days and 5-years), two Rhesus Macaques (24-days and 12-years) (Yerkes National Primate Research Center, Atlanta, GA); calf (10-months), sheep (3-years), lamb (4-months) (Innovative Research, Southfield, MI); cat, dog (Tuscawilla Oaks Animal Hospital, Oviedo, FL); cow, horse (HemoStat Laboratories, Dixon, CA); deer (Charles R. Daniels, DeLand, FL); spider monkey (Coriell Cell Repository, Camden, NJ); African crown cranes (2- and 3-years), gopher tortoise (20-years), and patagonian cavy (1-year) (Wuesthoff Reference Laboratory, Melbourne, FL). One buccal swab from a Chinese Muntjac (12-years) (Wuesthoff Reference Laboratory, Melbourne, FL) was also tested for specificity. All stains were stored at -45ºC until needed.

Fetal tissue (brain, heart, kidney, liver, spleen, thymus) and adult tissue (adipose, bone marrow, brain, kidney, liver, lung, skeletal muscle, skin, salivary gland, spleen, thymus, testis, uterus) total RNA was obtained from BD Biosciences Clontech (Palo Alto, CA). Fetal liver, fetal spleen, adult adipose and adult bone marrow DNA was obtained from BioChain Institute, Inc. (Hayward, CA).

RNA Isolation

A guanidine isothiocyanate-phenol:chloroform extraction method was used [22, 44, 53]. Briefly, 500-µL denaturing solution (4M guanidine isothiocyanate, 0.02M sodium citrate, 0.5% sarkosyl, 0.1M β-mercaptoethanol) was preheated in a Spin-Ease™ extraction tube (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD) at 56ºC for 10 minutes. Prepared stains were then added and incubated at 56ºC for 30 minutes. The stain was removed into a Spin-Ease™ extraction tube filter insert, placed back inside the extraction tube and centrifuged for 5 min at 16,000g, after which the filter and the fabric remnants were discarded. Fifty microliters of 2 M sodium acetate and 600-µL of acid phenol:chloroform 5:1, pH 4.5 (Ambion Inc., Austin, TX) were added to the extract, and incubated at 4ºC until two phases were resolved (~20 minutes), then centrifuged at 16,000g for 20 minutes. The RNA-containing aqueous phase was transferred to a sterile microcentrifuge tube, along with 30-µg GlycoBlue™ glycogen carrier (Ambion Inc., Austin, TX) and precipitated with 500-µL isopropanol overnight, at -20ºC. Samples were then centrifuged at 16,000g for 20 minutes to pellet the RNA. The supernatant was carefully removed and the pellet washed once with 1-mL 75% ethanol/25% DEPC-treated water and re-centrifuged at 16,000g for 10 minutes. The supernatant was discarded, the pellet dried in a vacuum centrifuge for 3-5 minutes and re-solubilized in 12-17-µL of RNAsecure Resuspension Solution (Ambion Inc., Austin, TX) at 60ºC for 10 minutes. RNA samples were treated with DNase I immediately or subsequent to storage at -20ºC.

DNA Isolation

Genomic DNA was extracted from 50-µL bloodstains by an organic solvent extraction method [54]. Briefly, samples were incubated overnight at 56ºC in stain extraction buffer (0.1M NaCl, 10mM Tris–HCl pH 8.0, 25mM EDTA pH 8.0, 20mM SDS) supplemented with 0.5 mg/ml proteinase K. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, pH 6.6) was added to the extract, mixed gently by inversion and centrifuged for 5 min at 16,000g to separate the phases. The DNA containing aqueous layer was transferred to a sterile microcentrifuge tube, precipitated with 1-mL of ice-cold 100% ethanol overnight, centrifuged at 16,000g for 15 minutes, washed once with 1-mL 70% ethanol and solubilized in 100-µL TE^{-4} (10 mM Tris, 0.1 mM EDTA) at 56ºC overnight.

DNase I Digestion

Extracted total RNA was treated with two units of TURBO™ DNase (RNase-Free) (2 U/µL) (Ambion Inc., Austin, TX) at 37ºC for 1-2 hours. The TURBO™ DNase was inactivated at 75ºC for 10 minutes, the samples chilled on ice and then stored at -20ºC until needed [45, 46].

Quantification of Nucleic Acids

RNA was quantified using a sensitive fluorescence assay based upon the binding of the unsymmetrical cyanine dye RiboGreen® (Molecular Probes, Eugene, OR) [47]. The manufacturer's instructions were followed for the high-range assay, which detects

from 20-ng/mL to 1-µg/mL. Briefly, 200-µL assays comprised of 2-µL TURBO™ DNase treated RNA extract, 98-µL TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5, in nuclease-free water), and 100-µL 750 nM RiboGreen® reagent in a 96-well plate format. After RiboGreen® addition and a three minute incubation at room temperature protected from light, fluorescence emission at 535 nm (excited at 485 nm) was determined using a Wallac Victor2 microplate reader (Perkin Elmer Life Sciences, Boston, MA). RNA concentration was calculated using an appropriate standard curve as described by the manufacturer [47]. All RNA samples were diluted to 5ng/uL (saliva and buccal swabs were diluted to 10ng/uL) with nuclease-free water (Ambion Inc., Austin, TX).

DNA was quantified by electrophoresis on 1% agarose gels stained with ethidium bromide and compared to known reference standards. All DNA samples were diluted to 1 ng/µL with TE^{-4} (10 mM Tris, 0.1 mM EDTA).

Reverse Transcription (cDNA Synthesis)

For all blood and tissue RNA samples 6-µL of RNA (30-ng), and for saliva/buccal 6-µL of RNA (60-ng), was heated at 75ºC for 3 minutes, snap cooled. For the mixture study total RNA from three newborns (<24-hours) and three juvenile/adult females (16-, 22-, and 31-years) were combined in different ratio combinations (1:1, 1:5, 5:1, 1:10 and 10:1) to yield the 6-uL (30-ng) necessary for the amplification. To the RNA, 4-µL of a 10 mM dNTP mix (Applied Biosystems, Foster City, CA), 2-µL of 10X first-strand buffer (500 mM Tris–HCl pH 8.3, 750 mM KCl, 30 mM $MgCl_2$, 50 mM DTT), 2-µL Random

Decamer primers (50-µM), 20-units SUPERase-In™ RNase Inhibitor (20 U/µL) (Ambion Inc., Austin, TX), 100-units Moloney Murine Leukemia Virus-Reverse Transcriptase (100 U/µL) (Ambion Inc., Austin, TX) and nuclease-free water (Ambion Inc., Austin, TX) were added to yield a final reaction volume of 20-µL. For the (–RT) reaction tubes the Moloney Murine Leukemia Virus-Reverse Transcriptase was replaced with Nuclease-free water. Reaction mixtures were incubated at 42ºC for 1 hour and 95ºC for 10 minutes to inactivate the reverse transcriptase [48, 49].

Polymerase Chain Reaction

Amplimer sizes for all genes tested are included in **Table 16**. All amplification reactions were conducted in a total volume of 25-µL containing genomic DNA (2-ng) or mRNA/cDNA (3-ng) (except for HBG1 and HBG2 singleplex reactions which contained 5-ng mRNA/cDNA). A standard reaction mix containing 1x PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.125 mM each dNTP, 0.4 µM primers and 1.25-units AmpliTaq Gold™ DNA polymerase (5 U/µL) (Applied Biosystems, Foster City, CA). Nuclease-free water (Ambion Inc., Austin, TX) was added to yield the final reaction volume.

For the newborn mRNA duplex HBG1n1-S15 and HBG2n3-S15 RT-PCR reactions, 3ng of cDNA was amplified with the following changes to the standard reaction mix: 0.6 µM S15 primers and 0.05 µM HBG1n1 or 0.05 µM HBG2n3 primers. The ribosomal protein gene transcript, S15, was included as an internal positive control for the reverse-transcription and amplification reactions.

For the fetal and adult tissue amplifications as well as the recombination experiment all single-gene reactions contained 400nM each primer and duplex reactions were as follows: HBG1n1-GNAS and HBG1n2-GNAS consisted of 400nM-100nM primers, and HBG2n2-GNAS and HBG2n3-GNAS consisted of 200nM-400nM primers.

Standard PCR conditions were used for all amplifications and consisted of an initial incubation step (95ºC; 11:00) followed by repeating cycles of [denaturation (94ºC; 0:20), annealing (60ºC; 0:30), and extention (72ºC; 0:40)] with a final incubation of (72ºC; 10:00) [50, 51]. Amplification cycle numbers are as follows: singleplex HBG, HBG1 and HBG2 (32 cycles; 55ºC annealing); HBG1n1 and HBG2n3 (28 cycles); singleplex HBG1-GNAS and HBG2-GNAS (35 cycles), duplexes HBG1n1-GNAS2 and HBG1n2-GNAS2 (30 cycles) and duplexes HBG2n2-GNAS2 and HBG2n3-GNAS2 (28 cycles).

Cloning and Sequencing

Newly identified hemoglobin products were excised from agarose gels and purified using MERmaid® SPIN columns, which specifically isolate low molecular weight DNA products (10-200 bp) (Q-BIOgene, Carlsbad, CA). Purified products were cloned into TOP10F' One Shot® chemically competent cells using the TOPO TA Cloning® Kit (pCR®2.1-TOPO®) (Invitrogen, Carlsbad, CA). Positive colonies were isolated and plasmids purified using the RapidPURE™ Plasmid Mini Kit (Q-BIOgene, Carlsbad, CA). Plasmids which contained the inserted product were sent to Lark Technologies for sequencing analysis (Lark Technologies, Inc., Houston, TX).

Post Amplification Electrophoresis

PCR and RT-PCR amplified products were visualized on 4% NuSieve® GTG® Agarose gels (Cambrex Bio Science Rockland, Inc., Rockland, ME). Electrophoresis was carried out at 100V for 1.25 hours in TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer. Gels were stained with SYBR® Gold nucleic acid stain (Molecular Probes, Eugene, OR), visualized on the Omega10 Chemiluminescence Imaging System (MLTRA-LUM, Inc., Claremont, CA) and analyzed with ONE-Dscan 2.05, 1-D Gel Analysis Software for Windows (Scanalytics, Inc., Fairfax, VA).

Duplex Real-Time PCR (qPCR)

All primer and probe sequences are listed in **Table 17**. All qPCR assays were performed in a 25-µL total reaction volume consisting of a standard reaction mix containing: three nanograms of cDNA (blood, semen, vaginal secretions, menstrual blood and breast milk) or six nanograms of cDNA (saliva/buccal), 12.5-µL Taqman® Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.25-µM of each probe and nuclease-free water (Ambion Inc., Austin, TX).

For the newborn assays (≤4 months old), 0.6-µM (S15), and 0.1-µM (HBG1n1) or 0.05-µM (HBG2n3) primers were added to the standard reaction mix. For the newborn assays (<24 hours old), 0.9-µM S15 primer and 0.05-µM HBG1n1 primer or 0.05-µM HBG2n3 primer were added to the standard reaction mix.

Real-Time PCR reactions were carried out on a 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Amplification conditions consisted of: (1) 1 cycle

of 50˚C; 2:00 (2) 1 cycle of 95˚C; 10:00 (3) 40 cycles of 95˚C; 0:15 and 60˚C; 1:00. Data was collected at stage 3, step 2 (60˚C; 1:00). Delta cycle threshold (dCt) values were calculated by subtracting the Ct value generated from the newborn specific gene from the Ct value of the housekeeping gene (i.e. dCt = Ct (S15) – Ct (HBG1n1 or HBG2n3) [52]. Samples which fail to amplify the newborn genes are given a default HBG1n1 or HBG2n3 Ct value of 40.00 (the amount of qPCR cycles used).

Recombination Experiment

Tissue DNA (43-ng) and blood DNA (64-ng) was treated with five units of TURBO^{TM} DNase (RNase-Free) (2 U/µL) (Ambion Inc., Austin, TX) or tissue DNA (50-ng) and blood DNA (75-ng) was treated with 100-units of RNase I (100 U/µL) (Ambion Inc., Austin, TX) at 37ºC for 30 minutes. The DNase I enzyme was inactivated by incubation at 75ºC for 10 minutes.[45, 46] The RNase I enzyme was inactivated by the addition of 50-µL phenol/chloroform/isoamyl alcohol (25:24:1, pH 6.6). RNase I treated DNA samples were then centrifuged at 16,000g (5-min) and the aqueous phase transferred to a new tube. Only 1-µL of these DNA samples (untreated, +DNase I, + RNase I) was added to the PCR reaction.

Blood and tissue total RNA (250-ng) was treated with 250-units of RNase I (100 U/µL) (Ambion Inc., Austin, TX) at 37°C for 30 minutes and inactivated with 60-µL acid phenol:chloroform 5:1, pH 4.5 (Ambion Inc., Austin, TX). The samples were centrifuged (10 minutes at 16,000g) and the aqueous phase precipitated at -20ºC overnight, with 15-µg GlycoBlue^{TM} glycogen carrier (Ambion Inc., Austin, TX) and 50-µL isopropanol.

Samples were then centrifuged at 16,000g (20-min), the supernatant removed and the pellet washed once with 200-µL 75% ethanol/25% DEPC-treated water, re-centrifuged at 16,000g (10-min), the pellet was dried in a vacuum centrifuge, and finally re-solubilized in 10-µL of RNAsecure Resuspension Solution (Ambion Inc., Austin, TX) at 60ºC for 10 minutes. The RNA samples were then reverse transcribed (see above) and 2-µL added to PCR.

Telomere Length Analysis for Biological Age Determination

Sample Preparation

Human blood samples were collected from Florida Hospital (Orlando, FL) only after exemption from the Hospitals Institutional Review Board and in accordance with procedures approved by our University's Institutional Review Board (**APPENDIX H: UCF IRB FORM**). Bloodstains were made by dispensing 50-µL aliquots onto sterile cotton gauze, allowed to air-dry overnight at room temperature and stored at -45ºC until needed.

DNA Extraction

Genomic DNA was extracted from 50-µL bloodstains by an organic solvent extraction method [54] followed by Centricon Filter Purification (Millipore Corp., Bedford, MA). Briefly, samples were incubated overnight at 56ºC in stain extraction

buffer (0.1M NaCl, 10mM Tris–HCl pH 8.0, 25mM EDTA pH 8.0, 20mM SDS) supplemented with 0.5 mg/ml proteinase K. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, pH 6.6) was added to the extract, mixed gently by inversion and centrifuged for 5 min at 16,000g to separate the phases. The DNA containing aqueous layer was transferred to a prewet Centricon filter and purified by washing with 2-mL TE^{-4} (10 mM Tris, 0.1 mM EDTA) and centrifugation at 2000g. Finally, DNA was removed by inverted centrifugation at 1000g with 100-µL TE^{-4}.

DNA Quantification

The real-time PCR Human Quantifiler™ Kit (Applied Biosystems, Foster City, CA) was used for quantification. Extracted DNA was diluted to a final working concentration of 10ng/uL with TE^{-4}, after comparison to a standard curve which was generated by running DNA samples of known concentrations from 25 to 0.63 ng/uL [55].

Delta Cycle Threshold Determination by Real-Time PCR – SYBR Green I Assay

Primer sequences are listed in **Table 21**. All qPCR assays were performed with a standard reaction mix containing: 17.5 nanograms of DNA, 1x SYBR® Green I PCR Buffer containing Passive Reference 1, 25-mM $MgCl_2$, 12.5-mM dNTPs, and 1.25-U AmpliTaq Gold DNA Polymerase (5U/µL) (Applied Biosystems, Foster City, CA). $T_{10}E_{0.1}$ (1M Tris-HCl pH 8.0, 0.5M Na_2EDTA) was used to bring the final volume to 25-µL. Primer concentrations for the telomere amplification were 270-nM tel 1 and 900-nM

tel 2, while the single copy gene (36B4) amplification required 300-nM 36B4u and 500-nM 36B4d [56].

Real-Time PCR reactions were carried out on a 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Real-time PCR amplification conditions consisted of: (1) 1 cycle of 95˚C; 10:00 and either (2T) 22 cycles of 95˚C; 0:15 and 54˚C; 2:00 or (2S) 30 cycles of 95˚C; 0:15 and 58˚C; 1:00, for the telomere (2T) and single-gene (2S) amplifications, respectively. Data was collected at stage 2, step 2 (54˚C; 2:00 or 58˚C; 1:00). Each standard or DNA extract was performed in duplicate and average cycle threshold values were determined. The delta Ct calculation was determined by the difference in amplification rates of the single-gene, 36B4, to the telomere repeats, $dCt = S_{Ct} - T_{Ct}$.

Real-Time PCR Amplification of Telomeres – TaqMan Assay

Primer and probe sequences are listed in **Table 21**. Genomic DNA (10.0 nanograms) was amplified in a standard reaction containing: 1x Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 250-nM probe (tel 3 or tel 6), 500-nM primers (tel 1 and tel 2 or tel 4 and tel 5), and an additional 5.0-U AmpliTaq Gold DNA Polymerase (5U/µL) (Applied Biosystems, Foster City, CA). Nuclease-free water was used to yield the final reaction volume of 25-µL.

Real-Time PCR reactions were carried out on a 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Real-time PCR amplification conditions consisted of: (1) 1 cycle of 95˚C; 10:00 and (2) 40 cycles of 95˚C; 0:15 and 50˚C; 2:00.

Data was collected at stage 2, step 2 (50˚C; 2:00). The cycle number at which the amplification curve reaches a pre-set threshold, the cycle threshold (Ct) value, was plotted against the biological age of each individual tested.

STELA Telorette Ligation Reaction

Genomic DNA (200ng) was ligated with 0.9uM each telorette linker (**Table 21**) in six separate reactions containing 1x manufacturers ligation buffer and 10-units T4 DNA Ligase (USB Corp., Cleveland, OH) for 12-hours at 35°C [57]. The T4 ligase was inactivated by heating at 65°C for 15 minutes. Ligated DNA was re-purified using Centricon Filters and re-quantified using the Human Quantifiler Kit as described in the DNA Extraction and Quantification sections, respectively, and diluted to a final concentration of 1 ng/uL with TE^{-4}.

STELA PCR Amplification

Forward (XpYpE2) and reverse (teltail) primer sequences are listed in **Table 21**. Telorette ligated genomic DNA (3-ng) was amplified in a 25-µL reaction volume containing 1x PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.3 mM each dNTP, 0.5uM telomere primer XpYpE2, 0.5uM teltail primer, 2.5-units AmpliTaq Gold™ DNA polymerase (5 U/µL) (Applied Biosystems, Foster City, CA) and nuclease-free water (Ambion Inc., Austin, TX) [57]. PCR conditions for all six telorette amplifications consisted of an initial incubation step (95ºC; 11:00) followed by

35 cycles of [denaturation (94ºC; 0:15), annealing (65ºC; 0:30), and extention (68ºC; 10:00)] and a final incubation of (68ºC; 10:00) [50, 51, 57].

STELA Post-Amplification Electrophoresis

PCR amplified products were visualized on 1% Agarose gels. Electrophoresis was carried out at 170V for 1.5 hours in TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer. Gels were stained with SYBR® Gold nucleic acid stain (Molecular Probes, Eugene, OR), visualized on the Omega10 Chemiluminescence Imaging System (MLTRA-LUM, Inc., Claremont, CA) and analyzed with ONE-Dscan 2.05, 1-D Gel Analysis Software for Windows (Scanalytics, Inc., Fairfax, VA).

CHAPTER THREE: RESULTS AND DISCUSSION

Messenger RNA Profiling Analysis for Biological Age Determination

Generating a Candidate Gene List from the Hybridization Intensities on the Affymetrix GeneChip® by Two-Group Comparison Analysis

Human venous blood was obtained from nine different individuals; three newborn babies (<24-hours old), three adolescent teenagers (14 – 18-years old) and three elderly males (74 – 79-years old). Total RNA from each individual sample was extracted and sent to Expression Analysis (Durham, NC) for hybridization to the GeneChip® Human Genome U133 Plus 2.0 Array (HG-U133+2). Three two-group comparisons were performed; newborns vs. juveniles, newborns vs. elderly, and juveniles vs. elderly, to provide gene expression fold changes between developmentally extreme age groups. The initial list of 54,675 probe sets was screened and all two-group comparisons which exhibited detection *p*-values >0.05 were eliminated. In general, the lower the *p*-value the more likely the transcript will truly be present in the sample, and *vice versa*. The 1,627 remaining probe sets (all statistically significant with *p*-values ≤0.05) were narrowed by eliminating all probe sets with absolute adjusted fold changes less than 2.0. The absolute adjusted fold change is the calculated fold change from the control to the experimental group. This measurement eliminates all probe sets with "Absent" detection calls in both the baseline and experimental comparison groups [58]. The remaining 1,241 probe sets from the Affymetrix GeneChip® two-group comparison results are shown in

APPENDIX C: AFFYMETRIX COMPARISONS. Included in the comparison summaries are the control and experimental group signals, which provide an overall estimate of individual transcript abundances for each group, the absolute adjusted fold change, the *p*-value and the gene symbols.

Generating Candidate Genes from Our Knowledge in Biochemistry and Physiology, as well as Published Literature Searches

Potential age dependent areas of molecular based developmental changes were searched in the NCBI PubMed literature database. In theory, an investigation of basic, highly characterized developmental changes should produce a battery of genes, whose expression patterns and relative levels have been examined in specific tissues, under specific conditions. Candidate genes for newborns (birth – 3-months) and infants (4-months – 9-months) included identifying proteins which were specific to fetuses or fetal tissues [59-62], including fetal specific protein isoforms [59, 60] and cellular immune responses [63]. Pre-pubertal developmental changes were examined to develop candidate genes for toddlers (10-months – 3-years) and children (4-years – 12-years) [62]. One example is the *N*-methyl-D-aspartate receptor gene (GRIN1, with transcripts NR1-1, NR1-2 and NR1-3, GRIN2A and GRIN2B) whose expression is increased in pre-pubertal mammals [64]. All juvenile or adolescent (13-years – 18-years) candidates were derived from pubertal developmental changes, mainly hormones which regulate sexual maturation. Female and male hormones, receptors, and activators are known to be upregulated in this age group and include estrogen [65], testosterone [66], and various sex steroids or endorphins [65-78]. Adults or post-pubertal (19-years – 45-years)

individuals were thought to also express hormones, however the relative levels of expression was thought to be less then that of juvenile individuals, and quantitative gene expression analysis would allow these age groups to be differentiated. Potential adult specific candidates were derived from known protein isoforms including the p45 adult specific form of the AUF1/hnRNP D (AU-rich element RNA binding protein-1/heterogeneous nuclear ribonucleoprotein D) gene [60]. The amount of research that has been conducted on generalized human ageing and the majority of our tested literature candidate genes ~69% (143/208) were targeted towards the middle-aged (46-years – 64-years) and elderly (>65-years) age groups. Major research areas have shown that specific molecular changes are increased in older individuals. These areas include an increase in the production of DNA damage machinery [79, 80] and other factors which are induced upon increased oxidative stress [81] and generalized DNA damage [23, 82]. At the present time it is unclear how apoptosis affects ageing, however apoptosis has been implicated in numerous diseases, which have been shown to correlate with increased biological age [83, 84]. Additionally, it has been illustrated that increased bone loss is evident in older ages [85]. Finally, a large number of age related gene candidates were obtained by published literature which illustrated an alteration in gene expression patterns. Examples include increases in cyclins D1 and E [59], the insulin growth factor binding proteins [86-88], various pro-inflammatory mediators [89], the tumor suppressor genes p53 and p21 [59, 90, 91], regulators of telomere length [92], and other various factors regulating transcription and gene expression [59, 90, 93]. A list of all candidate genes tested, along with the NCBI gene description, Nucleotide Accession number, and target age group is included in **APPENDIX D: CANDIDATE GENE DATABASE**.

Initial Screening of 319 Potential Candidate Genes by RT-PCR Gel Based Gene Expression Profiling Analysis

To determine the expression profiles of the potential candidate age related genes, PCR primers were designed using Primer3 design software (http://frodo.wi.mit.edu/). The input DNA sequence was obtained from the NCBI Nucleotide database and all mRNA sequences were BLASTed against the human genome to identify precise exon/intron boundaries. Primers were then designed to land in separate exons for facile separation of DNA and mRNA species (**Figure 3**). **APPENDIX E: CANDIDATE GENE PRIMER SEQUENCES FOR RT-PCR** lists all individual candidate primer sequences.

Potential age correlated candidate genes are tested by a series of RT-PCR amplifications with an array of biological ages (n=4-10), ranging from 1-hour old newborns to elderly individuals. All available extracted mRNA blood samples (n=147) for candidate testing are shown in **Table 1**. Genomic DNA is amplified with every candidate gene, as an amplification control for RNA specific detection (**Figure 3**). **Figure 4** illustrates the basic protocol of candidate gene testing by RT-PCR analysis. Initially, a first-round 35-cycle PCR amplification reaction is performed and based on the obtained results; a candidate gene is either rejected outright or is passed into a second round of PCR amplification. After this first-round amplification reaction candidates can be rejected for two reasons. First, candidates that have no amplified mRNA/cDNA product, yet show amplification of the genomic DNA control are rejected, mainly because if more than 35-cycles are required for visual product amplification, it is inferred that the transcript is present at extremely low levels and for the main goal of forensic assay development, these genes would not be ideal. Second, candidates that amplify an

mRNA/cDNA and genomic DNA control product of the same size are rejected, due to the fact that mRNA specific detection cannot be verified. Candidates can successfully pass this initial round of testing by generating one of two possible expression profiles. First, a candidate can exhibit amplification which shows a pattern of differential expression, termed sporadic expression or secondly, a candidate gene can amplify product in all ages tested. Candidates which are amplified in all ages tested are passed onto the next round but the amount of PCR cycles is decreased. PCR is an end-point analysis method and at 35-cycles, some high abundance transcripts appear saturated in all samples, but may actually be present in different copy numbers in varying ages. Therefore, a decrease in the amount of PCR cycles may illustrate a subtle sporadic expression pattern between different ages. Based on the results obtained in the first-round of testing the second-round of screening consists of either amplification at 35-cycles or at a decreased cycle number, usually 30, and regardless of cycle number, all candidates are assayed with a new array of biological ages (n=8). Combined analysis of rounds-one and -two determine if a candidate has passed onto the third and final round of screening. To pass into the third-round of amplification a candidate must exhibit a pattern of expression which is differentially expressed between ages and consistently expressed within an age group. The final amplification reaction is performed with a wide array of mRNA samples (n>30), with the purposed of verifying that amplification of the target age groups is consistent and that there is no amplification in the non-target age groups or there is a measurable amount of differential expression between age groups. At this juncture candidates are either accepted and taken to the quantitative RT-PCR analysis platform or rejected due to sporadic amplification of the target age group, specifically, if an amplicon

is present in only some of the target samples, or if non-differential expression is observed throughout all biological ages.

Using the Affymetrix GeneChip® and PubMed literature searches, 319 potential candidate genes were tested using the protocol described above and illustrated in **Figure 4**. A summary of the RT-PCR expression results is listed in **Table 2**, where the amount and percentage of accepted and rejected candidate genes is arranged by target age group, either newborns, juveniles, adults, or elderly, and by their source of acquirement, either the Affymetrix GeneChip® or literature searches. Of the 319 initial candidates, a total of 26 (8.15%) were accepted as potential biomarkers of biological age determination. Of these candidates; nine were from newborns, seven from juveniles and ten were from the elderly age group. These genes and their expression profiles are: AFP (fetal liver), COL1A2 (5-year), FLJ20344a (1-hour and 86-years), HBE1 (<3-months), and LOC151194 (1-hour and >68-years) for newborns (**Figure 5**). Additionally, four hemoglobin transcripts, HBG1n1, HBG1n2, HBG2n2 and HBG2n3 were determined to be specific to newborn blood and are shown in **Figure 29**. Juvenile candidates: ASL (>84-years), PPOX (sporadic), PRL (7-months), SPTRX-1 (3-years), SPTRX-2 (7-months – 3-years), TBC1 (14-years – 15-years), and TEKT2 (7-months – 3-years) are shown in **Figure 6**; while the elderly candidates: AGGF1 (<15-years), CDC2 (5-year), IGFBP3 (>29-years), LOH11CR2A (>79-years), MAD1L1 (<5-years), PDCD6 (5-years – 41-years), POLM (<13-years), POLQ (<5-years and 91-years), PPARD (<5-years), and SRC (<45-years) can be seen in **Figure 7** (see **APPENDIX D: CANDIDATE GENE DATABASE** for individual gene descriptions). These candidate genes were then transferred to the real-time PCR platform, which is described in the next section.

Alternatively, of the original 319 candidates, 105 (32.92%) which originated from the Affymetrix GeneChip® and 188 (58.93%) of the literature candidates, were rejected for a total of 293 (91.85%) rejected candidates. Rejected Affymetrix and literature candidates are categorized in **Table 3A and 3B**, respectively. After the first-round of RT-PCR analysis 6.0% (19/319) and 15.4% (49/319) of the Affymetrix and literature candidates, respectively, were rejected because no amplified mRNA/cDNA product was detected (**Figure 8**), whilst 8.5% (27/319) and 0.9% (3/319) of Affymetrix and literature candidates, respectively, were rejected due to the mRNA/cDNA and genomic DNA product amplifying at the same molecular size (**Figure 9**). The majority of rejected candidates (40.1%) from amplification rounds-two and -three consisted of those, which, even after decreased cycle number, amplified an mRNA/cDNA product in all biological ages tested, with no apparent difference in expression levels, specifically, 11.6% (37/319) of Affymetrix, and 28.5% (91/319) of literature candidates (**Figure 10**). The final group of rejected candidates, 6.9% (22/319) Affymetrix and 14.1% (45/319) literature, were those which exhibited sporadic expression when multiple samples of the target age range were amplified (**Figure 11**). **APPENDIX F: CANDIDATE GENE RT-PCR RESULTS** lists all candidates with their corresponding accepted age groups or rejection categories.

Quantitative Real-Time RT-PCR Gene Expression Profiling Analysis for 23 Potential Age Specific Biomarkers

Only after a candidate gene has passed numerous gel based screening amplifications, with various aged RNA samples, was it taken to quantitative real-time

PCR analysis. Real-time PCR provides numerous advantages over the conventional PCR technique, including increased sensitivity, decreased processing time and decreased sample consumption, which is a major advantage in forensic assay development; additionally, the ability of biomarker multiplexing with the inclusion of an internal positive control (IPC) is possible. Real-time PCR primers are designed, along with a sequence specific minor groove binding (MGB) probe, using ABI Primer Express software (version 2.0.0). To inhibit signal fluorescence from genomic DNA, primers are designed to land in separate exons, while the sequence specific probe is targeted to bind directly on the exon/exon boundary (**Figure 12**). All candidate gene probes are 5′ 6-FAM labeled, while the housekeeping gene, S15, is 5′ VIC labeled, and all probes are 3′ labeled with a non-fluorescent quencher (NFQ). All qPCR primers and probes are listed in **APPENDIX G: CANDIDATE GENE PRIMER SEQUENCES FOR qRT-PCR**.

Only 23 of the 26 potential candidates were taken to real-time PCR. The newborn biomarkers, HBG1n2 and HBG2n2 were omitted from real-time assay development because two hemoglobin derived newborn candidates (HBG1n1 and HBG2n3) had previously been discovered and assays had already been developed (see the **Fetal Specific Isoforms** section). The alpha-fetoprotein (AFP) gene was not persued at the qPCR level, because, although it is specific for the fetal liver, it was not detected in newborn blood (**Figure 5**).

In connection with the RT-PCR screening method, a real-time PCR protocol is followed for candidate gene testing. Initially, all genes are amplified with a range of biological ages (n>10), from 1-hour old newborns to elderly individuals, along with a genomic DNA control and a non-template control (NTC); one that has nuclease-free

water substituted for the nucleic acid. This first reaction verifies if the primer and probe set are mRNA/cDNA specific, and if there is any primer/probe interaction, based on amplification results of the genomic DNA and NTC, respectively. Amplification of a variety of biological ages is important for two reasons; first, differential amplification of the target age group must be verified by examining the cycle threshold (Ct) values generated against the non-target ages and second, the cycle threshold baseline of the target age group must be determined.

Results from the initial round of qPCR rejected 14 of the 23 candidates. First, the polymerase mu subunit gene, POLM, was the only candidate rejected due to the threshold never being crossed in any biological sample (**Figure 13**). The other 13 candidates were rejected because of non-differential expression, specifically, there was no amplifiable difference between target and non-target age ranges. These rejected genes include CDC2, POLQ, SRC, LOH11CR2A, ASL, FLJ20344a, LOC151194, SPTRX-1, SPTRX-2, PPOX, TBC1, TEKT2, and PRL. The Ct values for the biological ages tested, for each candidate gene, are shown in **Table 4**, along with data charts graphing the generated Ct values against their corresponding biological ages **Figure 14**.

Four of the original 23 candidates that were taken to real-time PCR produced differential Ct values in the first-round of screening which allowed them to be pursued further. These genes AGGF1, MAD1L1, PDCD6, and PPARD, along with their first-round amplification results and Ct values are shown in **Figure 15** and **Table 5**. As illustrated in these figures and tables, the younger aged individuals generated lower Ct values in the AGGF1 and PPARD amplifications, and increasing biological age, yielded increased Ct values. With the MAD1L1 and PDCD6 candidates, lower Ct values were

also generated with younger individuals, however the results were ununiform, whereby some younger ages exhibited Ct values that were consistent with older aged individuals, >50-years.

Second-round qPCR screening consists of designing and developing duplex reactions, incorporating the IPC and subsequent testing of a wide array of biological ages (n=96). Analysis of duplex reactions is conducted by calculating delta cycle threshold (dCt) values for each biological age, where the difference in amplification efficiency is determined by subtracting the Ct value of the GOI from the Ct value of S15, (dCt = Ct S15 – Ct GOI). This dCt metric signifies the amount of GOI transcript number in differing ages. To explain, all ages will have similar copies of the housekeeping gene and therefore should generate relatively similar Ct values for S15, acting as the IPC. In contrast, amplification of the GOI is dependent on the initial amount of transcript copy number, whereby target and non-target biological ages will have more and less copies of the GOI, thereby generating lower and higher Ct values, respectively. We can then compare the dCt values of unknown samples to those generated from known ages to determine biological age.

All four candidate genes, although producing differential amplification results in singleplex reactions, yielded blanket dCt values in duplex reactions. As illustrated in **Figure 16** and **Table 6** the results for AGGF1, MAD1L1, PDCD6, and PPARD, showed positive dCt values in all biological ages tested, from 1-hour to 102-years. These candidates were then rejected because of non-differential amplification of the GOI in the qPCR duplex reactions.

Discussed in the following sections are three candidate genes namely- COL1A2, HBE1 and IGFBP3, which showed target age specificity and were optimized into duplex reactions with the IPC housekeeping gene, S15. Additionally, two newborn candidates, HBG1n1 and HBG2n3, have been optimized into duplexes with the S15 housekeeping gene and validation studies have been completed (see the **Fetal Specific Isoforms** section).

COL1A2, A Biomarker for Age Determination of Younger Aged Individuals

When searching for literature candidate genes, an article by K. Kerschan-Schindl *et al*., reveled that the c-terminal telopeptide of type I collagen, had been increased in elderly subjects [85]. This lead to an investigation of all genes known to be associated with bone development; where the Homo sapiens collagen, type I, alpha 2 (COL1A2) gene, was identified as a potential biomarker of ageing. First-round singleplex amplification results illustrated that COL1A2 expression was increased in younger individuals, 1-hour to 12-years old, and all other ages >12-years, generated undetermined Ct values (default value of 50.000, the amount of qPCR cycles used) (**Figure 17** and **Table 7**). A duplex reaction was then optimized and amplification results with all biological ages (n=96) is illustrated in **Figure 18** and **Table 8**. The dCt values demonstrate that the amplification of the GOI is at a higher level in younger biological ages (1-hour – 5-months), when compared to other individuals (>5-months in biological age) due to the production of positive and negative dCt values, respectively. These results

established the development of a forensic tool for biological age estimation of younger aged individuals.

Initial validation specificity results, where 109 different blood samples were amplified in triplicate, are shown in **Table 9**, where each sample is listed with its corresponding average COL1A2 (GOI) and S15 Ct value and their standard deviations, as well as the calculated dCt values (±SD). **Figure 19**, gives a summary of the target age group (newborns and infants), in comparison to the non-target ages: toddlers, children, juveniles, adults, middle-age, and elderly individuals. Additionally, the overall average COL1A2 and S15 Ct values ±SD, and average dCt values are illustrated; specifically, the newborn and infant age group dCt value was +6.748 (±4.185 SD), compared to –2.980 (±5.020) in toddlers and children, and –4.830 (±3.681) in juveniles, adults, mid-age and elderly individuals.

HBE1, A Biomarker for Age Determination of Newborns

The Homo sapiens hemoglobin, epsilon 1 gene (HBE1), was selected as a potential newborn specific gene, due to its restricted expression to embryonic blood and select embryonic organs. First-round singleplex amplification results illustrated that HBE1 expression was increased in younger individuals, specifically 1-hour old newborns, and all other ages >17-days, generated at a minimum a 4 cycle higher Ct value (**Figure 20** and **Table 10**). A duplex reaction was then optimized and amplification results with all biological ages (n=96) is illustrated in **Figure 21** and **Table 11**. The dCt values demonstrate that the amplification of the GOI is at a higher level in younger

biological ages (1-hour – 3-months), when compared to older individuals (>3-months in biological age) due to the production of positive and negative dCt values, respectively. Although, some of the biological ages from 21-years to 92-years also positive dCt values, there is a detectable difference in the magnitude of positive dCt values, between the younger and older age groups.

Initial validation specificity results, where 139 different blood samples were amplified in triplicate, are shown in **Table 12** and **Figure 22**. Each sample is listed with its corresponding average HBE1 (GOI) and S15 Ct value and their standard deviations, as well as the calculated dCt values (±SD). **Figure 22** gives a summary of the target age group (newborns), in comparison to the non-target ages: infants, toddlers, children, juveniles, adults, middle-age, and elderly individuals. Additionally, the overall average HBE1 and S15 Ct values ±SD, and average dCt values are illustrated; specifically, the newborn age group dCt value was +3.088 (±2.523 SD), compared to –2.978 (±0.302) in infants, toddlers, children, juveniles, and adults; and –1.348 (±0.330) in mid-age and elderly individuals.

IGFBP3, A Biomarker for Age Determination of Post-Pubertal Individuals

The Homo sapiens insulin-like growth factor binding protein 3 gene, was identified when literature searches of human ageing reveled that mutations in Lamin A were responsible for prematureing ageing and that the levels of IGFBP3, decreased with lamin A splicing inhibition [82]. Additionally, a separate publication by Wang *et al.*,

listed IGFBP3, in addition to numerous others, as a gene with increased expression in senescent cells [59].

First-round singleplex amplification results of IGFBP3 illustrated that Ct values were obtained with all samples in the post-pubertal (>15-years old) age range (19/19), while IGFBP3 was unamplifiable in 71% (5/7) of samples aged 1-hour to 12-years old **Table 13**. **Figure 23** shows this initial round of amplification results, where undetermined +RT Ct values are given a default value of 40.000, the amount of qPCR cycles used.

After the favorable singleplex amplification results, a duplex reaction was optimized and amplification results with all biological ages (n=96) is illustrated in **Figure 24** and **Table 14**. The dCt values demonstrate that amplification of IGFBP3 (the GOI) is at a higher level in older biological ages, due to the production of positive dCt values in ages greater than 35-years old. More importantly, 96% (44/46) of the younger biological ages, those from 1-hour to 34-years old, produced negative dCt values. Only two samples a 14- and a 24-year old, produced positive dCt results of +2.126 and +2.054, respectively. These results established the development of a forensic tool for biological age estimation of post-pubertal individuals, aged 35-years and older. Initial validation specificity results, where 123 different blood samples were amplified in triplicate, are shown in **Table 15**. Each sample is listed with its corresponding average IGFBP3 (GOI) and S15 Ct values and their standard deviations, as well as the calculated dCt values (±SD). **Figure 25** gives a summary of the target age group (adults, middle-aged and elderly), in comparison to the non-target ages: newborns, infants, toddlers, children and to a lesser extent juveniles. The overall average IGFBP3 and S15 individual Ct values ±SD, are listed for each age group

and the calculated average dCt values are illustrated. Individual results from the IGFBP3-S15 duplex amplification yielded an average dCt value of –0.725 (±2.637), in the target age groups, compared to –9.914 (±5.402) in newborns, infants, and toddlers, and –4.208 (±4.260) in the children and juvenile age range.

Fetal Specific Isoforms of Gamma Hemoglobin as Biomarkers for Biological Age Determination

Expression Analysis of the Standard Hemoglobin Gamma Transcripts, HBG1 and HBG2

The tetrameric fetal and adult hemoglobin protein complexes are composed of two alpha and either two gamma ($\alpha_2\gamma_2$) or two beta ($\alpha_2\beta_2$) hemoglobin chains, respectively. This well characterized variation in fetal versus adult hemoglobin was the basis for the initial design of a newborn specific assay. The gamma hemoglobin locus was analyzed by a reverse transcription-polymerase chain reaction (RT-PCR) using three different sets of primers. The universal set amplifies both gamma hemoglobin genes simultaneously (HBG), while two sets of gene specific primers amplify either the HBG1 (A-gamma) or HBG2 (G-gamma) genes individually (**Figure 26**). All forward and reverse primers were designed to land in exons two and three (flanking intron two), respectively, for separation of cDNA and genomic DNA amplified products in agarose gels. To test the expression of the gamma hemoglobin transcripts over different biological ages, total RNA was extracted from venous bloodstain samples donated from individuals aged 1-hour to 91-years. Messenger RNA was reverse-transcribed and the

corresponding cDNA, along with a genomic DNA control, was amplified using primers designed to specifically recognize total hemoglobin (HBG) or the individual HBG1 or HBG2 gene transcripts (**Figure 27**).

Contrary to the initial hypothesis, a gamma hemoglobin messenger RNA amplified product corresponding to total HBG (154 bp) (**Figure 27A**) or individual HBG1 (277 bp) (**Figure 27B**) or HBG2 (274 bp) (**Figure 27C**) genes was amplified in all ages tested. Detection of these fetal hemoglobin chains ($^{A}\gamma$ and $^{G}\gamma$) in non-newborn blood samples was unexpected, based on our knowledge that expression of the fetal hemoglobin protein falls to ~3% within five months after birth and is completely replaced by adult hemoglobin after two years of biological age [35, 94]. These results demonstrate that, in contrast to the expression pattern for the fetal hemoglobin protein, HBG mRNA production is not solely restricted to the fetal and newborn stages of development. While evaluating the results from the standard hemoglobin amplification reactions in **Figure 27**, we serendipitously detected additional lower molecular weight bands in only the younger aged individuals aged 1-hour, 13-days and 3-months (+RT) (illustrated with asterisks) at approximately 65bp and 100bp, for the HBG1 (**Figure 27B**) and HBG2 (**Figure 27C**) amplifications, respectively.

Sequence Determination and Alignment of the Newborn Specific Gamma Hemoglobin Isoforms

To determine the molecular sequence of the newly identified low molecular weight amplimers; amplified products were excised from agarose gels, purified, cloned into chemically competent cells and sequenced (see **Cloning and Sequencing**). The

sequencing results for the HBG1 and HBG2 low molecular weight products revealed that each band was actually composed of two separate amplimers, which seemed to be of the same size or only slightly different. Once all four low molecular weight amplimers had been sequenced, we utilized the (NCBI) human genome BLAST alignment tool to determine the origin of the amplified products. Alignment results illustrated that both low molecular weight sequences obtained from the HBG1 reaction and both low molecular weight sequences from the HBG2 reaction, only aligned with regions of the original HBG1 and HBG2 transcripts, and did not exhibit sequence similarity to any other part of the human genome. The specificity of the forward and reverse primers for both hemoglobin genes was also tested using the NCBI nucleotide BLAST (search for short, nearly exact matches). The results illustrated that although the primers are not human specific, they are specific for the HBG1 and HBG2 transcripts, within the human transcriptome.

After determining that these amplimers originated from the standard hemoglobin gamma genes, MegAlign software from DNAstar Lasergene was used to align the lower molecular weight sequences to the corresponding standard hemoglobin sequences. This was necessary in order to determine the regions of similarity and dissimilarity within the transcripts. Alignment analysis showed that all four of the low molecular weight amplimers contained identical sequences to the standard hemoglobin sequences, beginning with the forward primer binding site (located in exon two) and extending to the reverse primer binding site (located in exon three). More importantly, alignment analysis illustrated that the middle of the amplified sequence was deleted in all four low molecular weight products, specifically, the 3′ end of exon two and the 5′ end of exon three, was

missing from all four of the sequences. Further evaluation of each specific deleted region (all four transcripts had a deleted region, however the size of the deleted region as well as the first and last nucleotides in the deletion was different) exhibited the presence of either a penta- or octanucleotide direct repeat sequence at the beginning and the end of each deletion. These direct repeat sequences were located in both exons two and three of each transcript and seemed to be the breakpoints between the aligned regions within the standard hemoglobin sequences and each of the four low molecular weight sequences. **Figure 28** illustrates the exact locations of these direct repeat breakpoints for the HBG1 and HBG2 genes and their low molecular weight products. Direct repeat sequences and their locations within the gene for HBG1 [Genbank: NM_000559] are ATGAT (292-296, 509-513) and AGATGCCA (272-279, 486-493), and the corresponding low molecular weight amplimers have been named HBG1n1 and HBG1n2, respectively. The direct repeats for the HBG2 [Genbank: NM_000184] gene are TGCCC (311-315, 473-477) and CACTG (330-334, 492-496) and the corresponding low molecular weight amplimers have been named HBG2n2 and HBG2n3, respectively. After the sequencing and alignment results were interpreted, the deleted regions and amplicon sizes for each of these transcripts was determined. For HBG1n1, HBG1n2, HBG2n2 and HBG2n3 the number of deleted bases was 217, 214, 162, and 162 bp, which produced amplicons of 60, 63, 112 and 112 bp, respectively. It should also be noted that although the sequence of the breakpoints is known, the actual position within the direct repeat where the break occurs is unknown.

RT-PCR Amplification of the Individual Newborn Gamma Hemoglobin Isoforms

Based on the sequencing results for the four newborn transcripts, gel based RT-PCR assays were developed for amplification of the individual transcripts. Forward primers for the HBG1n1, HBG1n2, HBG2n2 and HBG2n3 assays were designed to span the breakpoints in each of the two isoforms, therefore precluding the amplification of the standard HBG genes (**Table 16**, underlined sequences). Total RNA from bloodstains from three individuals aged 8-days, 15-years and 84-years were tested, along with a genomic DNA control. As expected an amplified product consistent with the detection of the four transcripts was detected only in the 8-day old newborn (**Figure 29**).

An internal positive control (IPC), the ribosomal protein, S15, was incorporated into two of the newborn assays resulting in two duplex RT-PCR reactions. S15 was chosen as the IPC instead of either of the commonly-used housekeeping genes, GAPDH or Beta-Actin, since S15 exhibited significantly fewer processed pseudogene derived artifacts in RNA isolates containing trace quantities of genomic DNA (data not shown). Each duplex reaction contained primers for the housekeeping gene, S15 [51], and one of the two newborn gamma isoforms, either HBG1n1 and HBG2n3. The S15-HBG1n1 (**Figure 30A**) and S15-HBG2n3 (**Figure 30B**) duplexes demonstrated the presence of S15 mRNA in all ages tested, while the newborn gamma hemoglobin gene transcripts were only found in individuals aged 1 hour to 3- and 4-months, respectively.

Quantitative Real-Time PCR Analysis of the HBG1n1 and HBG2n3 Newborn Specific Gamma Isoforms

The two gel-based duplex RT-PCR assays for the identification of HBG1n1 and HBG2n3 were re-configured for analysis using a real-time, quantitative PCR (qPCR) platform. The resulting prototype qRT-PCR assays as formulated, should detect the HBG1n1 and HBG2n3–derived amplicons at a significantly higher level in newborn individuals (≤4 months) compared to those of older age groups (>4 months). In order to accomplish this, the amount of HBG1n1 and HBG2n3 expression in different age groups was characterized by a dCt metric [Ct (S15) – Ct (HBG1n1 or HBG2n3)] which measures the expression of HBG1n1 and HBG2n3 isoforms in relation to the S15 internal positive control. Samples from newborns (≤4 months) typically generated Ct (HBG1n1 and HBG2n3) values less than that of S15, indicating the relatively high level of expression of HBG1n1 and HBG2n3 in newborns compared to the S15 housekeeping gene (**Figure 31A** and **Figure 31B**, left panels). In contrast, Ct (HBG1n1 and HBG2n3) values from non-newborns (>4 months old) were greater than generated S15 Ct values (**Figure 31A** and **Figure 31B**, right panels). In some non-newborn individuals (>4 months old) the HBG1n1 and HBG2n3 transcripts were present in insufficient quantity to reach the Ct threshold (**Figure 31B2**). In these instances the Ct (HBG1n1 and HBG2n3 was given a default value of 40.00 which represents the total number of PCR cycles used (i.e. 40). Therefore, the qRT-PCR assays, as configured, should produce positive dCt results for newborn blood samples whereas all other age groups should produce negative dCt results. For example, the dCt values of the newborns illustrated in **Figure 31A** and

Figure 31B were +1.60 (HBG1n1) and +5.60 (HBG2n3) whereas the non-newborns were –2.61 (HBG1n1, 72-years old) and –8.03 (HBG2n3, 15-years old).

In certain circumstances (e.g. newborns whom have been illegally removed from the hospital) it would be useful to determine whether an individual was <24 hours old. It was possible (see below), by altering the primer concentrations, to modify the two duplex qRT-PCR assays described above such that they were predictive (i.e. based upon a positive dCt metric) of blood from a child <24 hours old (**Figure 31C** and **Figure 31D**). Examples of the results from the <24 hours newborn assays are provided in **Figure 31C** (HBG1n1) and **Figure 31D** (HBG2n3). The corresponding dCt values for a 1-hour newborn were +3.75 (HBG1n1) and +5.89 (HBG2n3), whereas an 8-day old newborn produced values of –1.40 and –1.66, respectively.

The precise Ct that an amplified gene product attains is dependent on two factors, the amount of target gene present in the sample and the concentration of primer and probe used in the PCR reaction. The two newborn duplex real-time PCR assays (≤4 months and <24 hours) illustrate the effect these two factors have in real-time PCR amplification (**Figure 32**). Ubiquitously expressed genes (i.e. housekeeping genes) are expressed at relatively the same levels in all cell types. Differentially expressed genes have regulated expression patterns and are either turned on/off (i.e. present/not-present) or are expressed at different levels (i.e. increased/decreased) in a tissue or developmental stage specific manner. In both newborn assays the concentration of the newborn gamma hemoglobin isoform primers is similar (HBG1n1= 100nM (≤4 month) and 50nM (<24 hour); HBG2n3= 50nM (≤4 month) and 50nM (<24 hour)). Therefore, since the primer/probe concentrations are the same in both assays amplification of these

hemoglobin isoforms is dependent on the initial gene copy number. **Figure 32**, illustrates that a sample amplified with all four duplex reactions from both assays should produce a relatively constant HBG1n1 and HBG2n3 Ct value. Since the S15 housekeeping gene exhibits a constant level of expression (constant initial copy number), all samples should reach the threshold at relatively the same cycle number. Thus, increasing or decreasing the S15 primer/probe concentration will shift all amplification response plots to the left or right, respectively. The S15 primer concentrations vary significantly between the two newborn assays. In the ≤4 month assay 600nM is used, compared to 900nM in the <24 hour assay. Therefore, by increasing the S15 primer concentration all amplification plots (Ct values) are leftward shifted in the <24 hour assay when compared to the ≤4 month assay, and with the hemoglobin genes remaining constant, this allows older newborns to now produce negative dCt values (more S15 product than HBG1n1 and HBG2n3) when compared to younger newborns (more HBG1n1 and HBG2n3 product than S15) (**Figure 32**).

Biological Age Specificity of the qPCR Newborn Hemoglobin Biomarkers

The ability of the qRT-PCR assays to identify newborn individuals (≤4 months or <24 hours) was tested by analyzing 132 blood samples from multiple donors varying in biological age from 1-hour to 92-years (<24h (n=10); 1 day-1 month (n=19); 2-4 months (n=22); 5 months-3 years (n=37); 4-18 years (n=20); 19-92 years (n=24)). The results are summarized in the form of two-dimensional scatter plots in which each sample's dCt (S15-HBG1n1) and dCt (S15-HBG2n3) are displayed (**Figure 33**). Positive results from

newborns are expected to be confined to the upper right quadrant (positive dCt HBG1n1 and dCt HBG2n3) whereas negative results from non-newborns would be found in the lower left quadrant (negative dCt HBG1n1 and dCt HBG2n3).

In the ≤4 month assay, 98% (i.e. 50) of the 51 newborn (≤4 month old) samples yielded at least one positive dCt value, while 96% (i.e. 78) of the 81 non-newborns yielded two negative dCt values (**Figure 33A** and **Table 18**). Indeed the vast majority of ≤4 month old newborn samples (90% (46/51)), gave two positive dCt values (upper right quadrant). The one non-newborn sample that appears in the upper right quadrant in **Figure 33A** originates from a 7-month old infant. Subsequent repeat analysis (x2) places it in the lower right quadrant (i.e. one positive and one negative dCt). Of the samples that generated one positive and one negative dCt value, four of the six individuals were 4-months old. This is consistent with the occurrence of a transitional developmental state that occurs about 4-months after birth in which transcription of the HBG1n1 and HBG2n3 isoforms is curtailed.

For the <24 hour assay, all newborn samples aged from 1-hour to 24-hours generated positive dCt values (10/10) for each duplex (**Figure 33B** and **Table 18**). Ninety-seven percent (100/103) of individuals biologically aged greater than one-month generated two negative dCt values as expected.

No sex-specific differences were observed with either the <24 hour or ≤4 month assays (data not shown).

Body Fluid Specificity of the qPCR Newborn Hemoglobin Biomarkers

Saliva (n=18), semen (n=2), vaginal secretions (n=2) and menstrual blood (n=7) from healthy donors; as well as venous blood, saliva, vaginal secretions from a pregnant female and breast milk (1-month post delivery) were assayed with the ≤4 month and <24 hour duplexes. HBG1n1 and HBG2n3 Ct values were undetermined for all body fluids tested, illustrating that the two duplexes are specific for venous newborn blood (**Figure 34**).

Human Specificity of the qPCR Newborn Hemoglobin Biomarkers

RNA was extracted from bloodstains from a variety of animal species including two Pigtailed Macaques (one newborn and one adult), two Rhesus Macaques (one newborn and one adult), calf (newborn), cow (adult), lamb (newborn), sheep (adult), cat, dog, horse, deer, spider monkey, two African crown cranes, gopher tortoise, and a patagonian cavy, and tested with the newborn qPCR assays. One buccal swab from a Chinese Muntjac was also tested. HBG1n1 and HBG2n3 cycle threshold (Ct) values were undetermined for all animal samples tested, illustrating that the two duplexes are specific for human newborn blood (**Figure 35**).

Mixture Study of the qPCR Newborn Hemoglobin Biomarkers

The newborn assays are expected to be of use in the investigation of criminal abortion cases. In such instances putative products of conception are sometimes recovered and expected to comprise mixed samples, typically the newborn (or fetus) and

that of an adult. Therefore, to ensure the detectability of newborn blood in the presence of adult blood, controlled mixture studies were carried out. Total RNA from the blood of newborns (<24-hours old) and either juvenile (16-years) or adult (22- or 31-years) individuals was combined to simulate mixtures from criminal abortion cases. Three separate newborn/non-newborn admixed pairs were studied with each pair comprising a sample set of the same admixture ratios (1:1, 1:5, 5:1, 1:10 and 10:1). The 15 mixed RNAs were reverse-transcribed and amplified with both newborn duplexes, using the ≤4 month and <24 hours assay formats.

In the ≤4 month assay all 15 mixtures generated two positive dCt values, except for one of the 1:10 mixtures (24-hour newborn: 31-year adult) (**Figure 36A**). This latter sample generated one positive (S15-HBG1n1) and one negative (S15-HBG2n3) dCt value. In the <24 hour assay all of the 1:1, 5:1 and 10:1 mixtures generated two positive dCt values (**Figure 36B**). The three 1:5 mixtures and two of the three 1:10 mixtures generated one positive and one negative dCt value in the S15-HBG2n3 and S15-HBG1n1 assays, respectively. The other 1:10 mixture (24-hour newborn to 31-year adult) generated two negative dCt values (**Figure 36B**).

The above results indicate that the assays can detect newborn/non-newborn admixed samples and are likely to be of use to demonstrate the presence of newborn blood in putative products of conception.

Real-Time PCR Sensitivity of the Newborn Hemoglobin Biomarkers

The sensitivities of the qRT-PCR newborn assays were determined by varying the amount of total RNA input into the assays using RNA isolated from bloodstains from two newborns (both 1-hour old) and two non newborns (a 13-year old and a 53-year old). The average dCt values from both newborns and both adults are shown for each duplex reaction (**Figure 37**, **Table 19**, and **Table 20**). The ≤4 month newborn assay generated positive dCt values with ≥ 5 pg RNA with the newborn samples while the adult samples generated negative dCt values with ≥ 50 pg RNA (**Figure 37A** and **Table 19**). Input RNA less than these concentrations did not produce detectable housekeeping gene or newborn gene products that reached the Ct threshold. With the <24 hour newborn assay, the S15-HBG1n1 duplex generated the expected positive and negative dCt values (newborns and adults, respectively) with ≥ 25 pg RNA input. (**Figure 37B1** and **Table 20**). With the S15-HBG2n3 assay, newborns generated positive dCt values with ≥ 5 pg of input RNA, while the adult samples generated negative dCt values with ≥ 50 pg input RNA (**Figure 37B2** and **Table 20**).

Based upon these sensitivity studies, a minimum input of 50-pg RNA is recommended for the qRT-PCR newborn assays.

Stability of HBG1n1 and HBG2n3 Transcripts in Aged Bloodstains

In order to be useful in forensic casework, the HBG1n1 and HBG2n3 transcripts should be stable over time in dried stains. In order to assess the stability of the newborn transcripts in the dried state, blood from two newborns (1-hour and 2-months old), two

juveniles (14- and 15-years old) and two elderly individuals (84- and 86-years old) were deposited on cloth, allowed to air dry and stored at room temperature (~25˚C) for various time points (1, 3, 6, 9, 12 and 15 months). Total RNA was isolated from the bloodstains and then assayed for HBG1n and HBG2n transcripts by qRT-PCR. The results are displayed in a two dimensional scatter plot as before (**Figure 38**). In both newborn assays (i.e. ≤4 months and <24 hours), the one-hour old newborn individual generated two positive dCt values in all aged samples, while the juvenile and elderly individuals generated two negative dCt values at all time points tested.

Despite the excellent specificity exhibited by the assays with 15-month aged bloodstains (i.e. aged newborn bloodstains cluster separately from aged bloodstains from other developmental age groups), caution must be exercised in aged samples from older newborns. While the two-month old newborn sample produced two positive dCt values when stored at room temperature up to one month with the ≤4 month assay, it produced one positive and one negative dCt value for the S15-HBG2n and S15-HBG1n duplexes respectively with stains aged 3-15 months (**Figure 38A**). In the <24 hour assay, the same two-month old newborn produced one positive and one negative dCt value when aged for 1, 3, 6 and 9 months but two negative dCts after 12 and 15 months of storage (**Figure 38B**).

Expression Profiles of the Standard and Newborn Hemoglobin Gamma Transcripts in Six Fetal Tissues

With the discovery of four novel hemoglobin derived transcripts, each exhibiting regulated expression to only newborn blood, we next evaluated the expression patterns of

each of the hemoglobin genes (HBG1, HBG1n1, HBG1n2, HBG2, HBG2n2, and HBG2n3 (primer sequences are located in **Table 16**)) in total RNA obtained from six fetal tissues: brain, heart, kidney, liver, spleen, and thymus. Additionally, total RNA was extracted from three blood samples, donated by different biologically aged individuals and assayed as amplification positive (1-hour old) or negative (45- and 79-years old) controls. To correctly determine the expression patterns of the novel transcripts, RT-PCR duplex reactions were created by integrating the housekeeping gene, GNAS (guanine nucleotide binding protein, alpha stimulating), into amplification reactions with each of the individual hemoglobin transcripts. Incorporating the housekeeping gene as an internal positive control (IPC), allowed us to conclude if the hemoglobin transcripts were truly present or truly absent in each particular tissue sample. By visualizing the positive amplification of the GNAS transcript, the quality and quantity of our RNA/cDNA sample, along with the reverse transcription and PCR reactions, were verified.

Figure 39 illustrates the expression profiles obtained from the duplex hemoglobin amplification reactions. Consistent amplification of the IPC (GNAS), in all blood and fetal tissue samples, allowed us to directly compare the expression patterns of the hemoglobin genes. Results obtained from amplification of the standard hemoglobin HBG1 and HBG2 transcripts (**Figure 39A** and **Figure 39D**), illustrate that all blood and tissue samples showed nearly identical amplification products, with no age related differences in gene expression. These results confirm our previous experiment of analyzing (and rejecting) the standard hemoglobin genes as potential newborn specific mRNA candidates (**Figure 27**). Although the expression of the HBG1 and HBG2 mRNA transcripts in non-newborn blood samples seem to contradict our current knowledge of

the expression of the fetal hemoglobin protein (i.e. HbF is found at levels <2% in normal adult blood) [95-97], the results do stimulate the possibility of a post-transcriptional regulation mechanism, triggered shortly after birth, which inhibits fetal hemoglobin protein synthesis.

In contrast to the uniform expression patterns obtained from the standard hemoglobin genes in blood and fetal tissue samples, the four novel hemoglobin transcripts seemed to have tightly regulated and individualistic expression patterns (**Figure 39**). All four transcripts were detected in the 1-hour old blood donor (+RT only), but were not amplified in either the 45- or the 79-year old donors, confirming their specificity to newborn blood. Amplification of the HBG1n1 and HBG2n3 genes was detected at high levels in fetal liver and fetal spleen and lower expression was seen in the fetal heart, kidney and thymus tissue, however neither gene was detected in fetal brain (**Figure 39B** and **Figure 39F**). The HBG1n2 transcript exhibited relatively low expression levels in fetal heart, liver and spleen tissues, and was not detected in fetal brain, kidney or thymus samples (**Figure 39C**). In contrast to the other three novel hemoglobin genes, the expression of the HBG2n2 transcript was restricted to the newborn blood sample, and was not detected in any of the fetal tissues (**Figure 39E**). The expression of the standard and novel gamma hemoglobin transcripts at higher levels in fetal liver and spleen was expected, due to the fact that the liver and to a lesser extent the spleen are the major sites for hematopoiesis, after the transition from vitelline to placental circulation and disappearance of the embryonic yolk sac [35].

Expression Profiles of the Standard and Newborn Hemoglobin Gamma Transcripts in Thirteen Adult Tissues

After determining the expression patterns of the newborn hemoglobin transcripts in fetal tissues, we next evaluated their expression in adult derived tissues. Total RNA from adult adipose, bone marrow, brain, kidney, liver, lung, skeletal muscle, skin, salivary gland, spleen, thymus, testis, and uterus was amplified, along with newborn blood (1-hour) as a positive control and two negative controls, an adult (45-years old) and an elderly (79-years old). Duplex RT-PCR reactions, containing the GNAS housekeeping gene and a newborn transcript of interest, were performed and the results were evaluated by agarose gel electrophoresis (**Figure 40**). All samples successfully amplified the GNAS control, which allowed us to directly compare the hemoglobin amplification expression patterns. All three blood samples, along with adult adipose and bone marrow exhibited high transcript levels when assayed for the standard HBG1 and HBG2 genes, **Figure 40A** and **Figure 40D**, respectively. In contrast, only the newborn blood (1-hour) positive control was amplified with any of the newborn specific hemoglobin gene duplexes, the two blood negative controls and all thirteen adult tissues failed to amplify with the HBG1n1, HBG1n2, HBG2n2 and HBG2n3 genes in **Figure 40B**, **Figure 40C**, **Figure 40E**, **Figure 40F**, respectively.

The four novel hemoglobin transcripts showed restricted expression, only being detected in newborn blood, which coincides with our knowledge of fetal hemoglobin protein expression. The presence of the fetal hemoglobin transcripts was expected in adult bone marrow, due to it being the primary site of hematopoiesis, taking over for the fetal liver at approximately 20 weeks gestation [35]. The presence of the HBG1 and

HBG2 transcripts in human adult adipose tissue was unexpected, due to its lack of direct association with erythropoiesis. A literature search identified a single report of the gamma hemoglobin promoter being active in adipose tissue. This study was conducted using transgenic mice which linked the human fetal HBG2 promoter to the viral simian virus 40 T antigen [98]. After transfection, the presence of brown adipose tumors was detected in the subcutaneous interscapular neck and shoulder areas; illustrating the ability of the gamma hemoglobin promoter to be transcribed in adult mouse adipose tissue [98].

Molecular Origin of the Four Newborn Gamma Hemoglobin Isoforms

Sequencing results from the four novel hemoglobin transcripts illustrated that an octanucleotide (HBG1n2) and varying pentanucleotide (HBG1n1, HBG2n2, HBG2n3) repeats were the initiation sites for the partial gene deletions (**Figure 28**). At the molecular level it appears that the newborn transcripts are formed by rearrangement events, which excise the intervening regions of exons two and three, between the repeated segments. **Figure 41A** illustrates a potential rearrangement event occurring at the genomic DNA level, while **Figure 41B** illustrates an interaction at the RNA level and although intermolecular interactions are illustrated, the potential for intramolecular rearrangements is inferred. A recent paper by Lutay *et al*, illustrated the ability of RNA, in nonenzymatic recombination reactions, to successfully create novel RNA molecules from two oligonucleotides, *in vitro* [99]. Because our four transcripts were detected at the RNA level, the possibility of an RNA based origin was evaluated, in addition to the recombination event originating at the genomic DNA level [22]. These two possibilities

were evaluated by extracting total genomic DNA and RNA from newborn (1-hour), adult (31-years) and elderly (86-years) bloodstains or purchasing genomic DNA and total RNA from fetal liver and spleen and adult adipose and bone marrow (BM) (See Materials and Methods). The extracts were treated with DNase I or RNase I digestions, followed by amplification with the previously described duplex PCR reactions containing the GNAS housekeeping gene and one of the four newborn specific isoforms (**Figure 42**). In all eight blood samples (**Figure 42A**), and fetal and adult tissues (**Figure 42B**), the genomic DNA extracts solely produced amplicons of the expected GNAS product size (855 bp) in the untreated and RNase I treated samples, lanes 1 and 3, respectively, with no amplification detected in the DNase I treated samples, lane 2. Additionally, none of the blood or tissue DNA samples, lanes 1-3, amplified a newborn hemoglobin isoform. For the RNA extracts, all samples were DNase I digested to eliminate any contaminating DNA before analysis. Lanes 4, 5 and 6, 7 represent total RNA treated or untreated with the RNase I enzyme, respectively. Following ±RNase I treatment, the RNA samples were reverse transcribed with or without the reverse transcriptase enzyme (±RT) and amplified with the duplex PCR reactions. The amplification results illustrate that all reverse transcribed RNA samples failed to amplify a GNAS or a hemoglobin product after treatment with both the DNase I and RNase I enzymes (lanes 4 and 5). In contrast, when total RNA was treated with the DNase I and RT enzymes (no RNase I digestion) we were able to detect an mRNA/cDNA product corresponding to the GNAS gene (371 bp, lane 6) in all samples tested, while only the newborn blood samples, fetal liver and fetal spleen amplified the newborn hemoglobin isoforms. The HBG2n2 gene did not amplify

in the fetal liver sample, which was expected due to it only being expressed in newborn blood (**Figure 39E**).

If the recombination event which leads to the generation of these four novel isoforms was occurring at the genomic DNA level we would expect to amplify a product for these transcripts in our untreated and RNase I treated DNA samples, in tandem with the GNAS positive control. Our results indicate that the hemoglobin transcripts are not found at the genomic DNA level. In RNA samples treated with DNase I and the RT enzyme, successful amplification of GNAS and the newborn hemoglobin isoforms is detected in newborn blood donors and fetal tissue origins. These results allow us to conclude that the recombination event which creates these novel transcripts is occurring at the molecular RNA level.

Telomere Length Analysis for Biological Age Determination

One highly studied molecular based degenerative process is the shortening of telomeric chromosomal regions with increasing chronological age [14, 100-106]. Telomeres are short tandem repeat sequences located at the ends of chromosomes and range from 100 to 280 nucleotides. They function to maintain chromosomal end integrity and stability by preventing exonuclease DNA degradation as well as inappropriate chromosomal fusions and by protecting the ends of linear chromosomes from being recognized by the DNA repair machinery as damage. The telomere repeat core sequence in humans is TTAGGG and is present at the tips of chromosomes both as a block of contiguous perfect repeats and, more distally, as a block of imperfect tandem repeats. A subtelomeric region comprising additional sequences separates the telomere from the rest of the chromosome. Telomeres play an essential role in DNA replication in that after each cell division a number of tandem repeats are lost due to the inability of replicative DNA polymerases to synthesize DNA in the 5′ to 3′ direction (**Figure 44**) [103, 107]. This "end replication problem" results in a loss of ~50-200 bp of DNA and a progressive reduction in telomere length and generation of a G-rich 3′ overhang [103, 107, 108]. The current paradigm is that the structural integrity of the telomere is regularly monitored by the cellular machinery and a number of telomere-specific protein sensors (e.g. TRF1, TRF2, POT1, TIN1, TIN2) have been identified [109]. Although telomerase is an enzyme with reverse transcriptase activity that can reconstitute the lost repeats, its expression is normally restricted to germ and stem cells. Thus somatic cells exhibit a progressive

reduced telomere length as cells divide and eventually the protective effect of the telomere structure is overcome and genomic instability or reproductive senescence results [110]. Progressive reduction in telomere length in somatic tissues is thus correlated with the 'biological age' of the cell and its use as a predictor of organismal 'chronological age' has been suggested [102, 109, 111].

Empirical observations in humans support the hypothesis that the average telomere length is inversely correlated with age [109]. Moreover reactive oxygen species (ROS) also cause telomere repeat loss and, since exposure to ROS is accumulative with age, telomere length might even be exacerbated in older individuals [112]. A number of other factors could potentially confound the use of telomere length. Nevertheless preliminary investigations by Ikeda and colleagues using bloodstains and teeth indicate that telomerase length estimation as an age indicator is possible with forensic specimens [14, 28].

The generally accepted approach for telomere length determination involves terminal restriction fragment (TRF) analysis, in which DNA is enzymatically digested and segments detected using Southern hybridization to a probe containing the telomeric repeat. However, this method offers low resolution and suffers from a lack of sensitivity, requiring approximately 0.5 - 1 μg of human genomic DNA or more than 10^5 cells, (an almost unobtainable quantity from the common 50-μL bloodstain normally encountered in forensic situations), as well as the reduced ability to detect shorter telomeres [109, 113]. In addition, TRF represents the mean telomere length of all chromosomes and includes the unknown length of the subtelomeric region, where the TRF value is dependent on the restriction site of the subtelomeric region by the restriction enzyme

[113]. Thus TRF does not provide information on actual telomere length. To overcome the downfalls of TRF analysis, two novel experimental approaches aimed at telomere length determination have been investigated. The first approach is based on real-time PCR amplification, and utilizes either the absolute quantification SYBR® Green I [56, 114] or the relative quantification Taqman® platforms, while the second, a single telomere length analysis (STELA), uses a novel telomere-telorette ligation reaction [57]. Both technologies can determine whether accurate, high resolution 'age of an individual' estimates can be made from dried biological stains.

Assessing Total Telomere Length by Delta Cycle Threshold Determination using Real-Time PCR and Telomere Specific Primers – SYBR Green I Assay

In real-time absolute quantitative PCR, detection of product is monitored by measuring the increase in fluorescence caused by the binding of the SYBR Green dye to double-stranded DNA [115, 116]. Quantitative PCR determines, for each sample well, the Cycle threshold (Ct) value, i.e. the fractional cycle number at which the well's accumulating fluorescence crosses a set threshold that is several standard deviations above baseline fluorescence [56, 117]. A recent paper by R. Cawthon measured the relative telomere length of an individual using a quantitative PCR approach [56]. This method of telomere length determination is based on measuring, for each DNA sample, the factor by which the "unknown" DNA sample differs from a reference DNA sample in its ratio of telomere repeat copy number (T), to a single copy gene number (S), thereby generating a final T/S ratio. The acidic ribosomal phosphoprotein PO gene, 36B4, was chosen as the single copy number gene due to its equal amplification in all DNA samples

tested [56, 118]. In theory, all samples should amplify the 36B4 single copy gene, at the same rate and hence, generate similar Ct values. In contrast, unknown DNA telomere lengths should vary between biological ages and therefore generate varying Ct values, corresponding to that specific samples telomere length (i.e. the younger the individual, longer the telomere, the lower the generated Ct value). The calculated difference in amplification rates between the telomere and single gene assays, the delta Ct ($dCt = S_{Ct} - T_{Ct}$), should reveal the quantity (length) of telomeres in relation to the single copy gene, assuming there is greater then one telomere present in each DNA sample. The calculated dCt values should then correlate with the biological age of the individual, whereby younger individuals generate larger dCt values, due to lower telomere Ct values; conversely, elderly individuals would generate smaller dCt values, because of increased telomere shortening. In our approach to relative telomere length determination, Ct values were generated according to the published primer and amplification specifications however; data interpretation consisted of calculating delta cycle threshold (dCt) values, instead of T/S ratios, for the standards and unknown DNA samples.

Genomic DNA was extracted from individuals of various ages ranging from hour old neonates to a 91-year old elderly individual. Real-time PCR amplification was performed in duplicate, for both serially diluted DNA standards and the unknown samples of various biological ages. The average telomere length (T) and single copy gene number (S) values were calculated and an average delta Ct value was determined (**Table 22**). **Figure 45** illustrates the dCt values for each diluted standard (50 to 6.3ng) and all biological ages assayed (1-hour to 91-years).

The single copy gene, 36B4, was amplified in all samples as an internal reference for the delta Ct calculations. **Table 22** illustrates that for the diluted DNA standards the 36B4 and telomere Ct values increase, in correlation with decreased input DNA. Delta Ct analysis of the standards verify the correlation of 36B4 and telomere amplifications, independent of input DNA, the dCt values are relatively similar, ranging from 0.875 to 1.620 (average 1.302 ± 0.2722). Analysis of the biologically aged blood samples revealed that the single gene amplification was consistent throughout, generating a range of Ct values from 26.580 to 32.240 (average 28.006 ± 1.4715), however, we did not detect any additional variation in the telomere amplification with Ct values ranging from 23.650 to 29.395 (average 25.224 ± 1.4629). **Figure 45** illustrates the calculated dCt values from the various biologically aged individuals and in contrast to our expectations of decreasing delta Ct values with increasing biological age, we actually find that the highest dCt value was generated by a 91-year old individual (dCt = 3.360), while a 45-year old generated the lowest dCt value of 2.050, while all other biological ages had dCt values in between the 45- and 91-year olds.

Telomere Length Determination by Real-Time PCR Amplification using a Telomere Specific Probe – TaqMan Assay

We developed a novel quantitative real-time PCR (qPCR) based assay for telomere length analysis of biologically aged individuals utilizing a TaqMan qPCR approach. This method utilizes a set of gene specific primers and a gene specific probe to determine the rate of amplification of a target gene sequence. Briefly, the fluorescently labeled oligonucleotide probe binds initially to the target DNA sequence due to its high

annealing temperature, followed by binding of the forward and reverse primers. As amplification occurs the probe is cleaved by the 5′ exonuclease activity of the polymerase enzyme, thereby releasing the fluorescent signal attached to the 5′ end of the probe, which is interpreted by the analysis software of the real-time PCR instrument. As amplification continues a Ct value (i.e. the fractional cycle number at which a samples accumulating fluorescence crosses a set threshold that is several standard deviations above baseline fluorescence) is generated [56, 117]. We designed two different primer/probe sets to specifically land-on and amplify the telomere repeats (**Table 23**). The first combination set of real-time primers and probe consisted of longer sequences (tel 1=37bp, tel2=39bp, tel3=31bp) and yielded a higher overall annealing temperature of 68ºC, when compared to a second primer/probe set (tel 4=25bp, tel5=27bp, tel6=19bp), which was shorter in length and annealed at a lower temperature of 53ºC (**Table 23**). Our thought for developing this type of approach was that all biological ages have telomeres and all ages would amplify the telomeres, however if telomere length is correlated with age, then younger individuals would have longer telomeres, which would yield more fluorescent signal (due to more potential sites for primer and probe binding) and these samples would reach the predetermined threshold value at a lower fractional cycle number when compared to individuals of increasing biological age.

Real-time PCR amplification of genomic DNA obtained from various biologically aged individuals yielded cycle threshold (Ct) values ranging from 8.438 to 11.243, primer set 1 and 5.261 to 6.911, primer set 2 (**Table 23**, **Figure 46**).

The results in **Table 23** and **Figure 46**, show that newborns and elderly individuals (1-hour, 89-years and 91-years) had the highest Ct values (mean = 9.75),

while all ages in between (4-, 14-, 47-, and 63-years) consistently generated lower Ct values (mean = 8.63). These results looked very promising, until we tested the primer/probe set with a non-template control (NTC) sample. It was shown that the NTC was able to generate a Ct value similar to the ones obtained with our DNA samples. It was determined that this amplification was due to primer and probe interactions, whereby the probe was binding to one of the primers and the TaqMan polymerase was cleaving the probe to release a fluorescent signal. Multiple reaction parameters were tested to try and overcome the NTC amplification, without success.

Assessing the Length of Individual Telomeres using the STELA Telomere Amplification Reaction

The single telomere length analysis (STELA) assay, has been developed for sizing the XpYp telomere, but has the potential of determining accurate telomere lengths for chromosomes 12q, 7q, 16q and 16p [57]. This method can successfully sizes telomeres, of all lengths, with as little as 250 pg template DNA. To describe the method briefly, six linker ('telorettes') comprising the seven bases with telomeric repeat homology (TTAGGG, TAGGGT, AGGGTT, GGGTTA, GGTTAG, and GTTAGG), followed by a 20-basepair segment (TGCTCCGTGCATCTGGCATC) non-complementary to the 3′ overhang are annealed and ligated to all telomere ends. The downstream 20-basepair non-complementary 'telorette' segment can then serve as a target for a PCR primer ('teletail'). Chromosomal specificity is generated by using an upstream primer which is designed to bind in the subtelomeric region of the target chromosome. Post-amplified fragments are electrophoresed and visualized with nucleic acid staining on agarose gels.

To determine the length of the XpYp telomere in varying biological ages, we extracted DNA from sixteen different individuals aged 1-day (male), 1-day (female), 4-months, 9-months, 15-months, 21-months, 8-years, 12-years, 17-years (male), 17-years (female), 29-years, 43-years, 56-years, 56-years, 91-years, and 92-years. We ligated each of the six linkers to the DNA samples and amplified each aliquot with the 'teltail' and XpYp chromosome specific primer.

The results obtained from these experiments did not allow us to conclude that telomere length is correlated with biological age, based on the following results (**Figure 47**). First, we found that there was inconsistent amplification of the samples between the linkers. For example, two of the linkers amplified fourteen of the sixteen samples (telorettes 1 and 6), one amplified twelve of the sixteen samples (telorette 3), while the three other linkers amplified only seven of the samples (telorettes 2, 4, 5). Second, the lengths of the amplified DNA samples did not correlate with the biological ages of the individuals. In linker two, a 4-month old individual had a longer telomere length then both of the 1-day old samples, and in linker three, the 91- and 92-year old individuals had longer telomeres then the 15- and 21-month old individuals. The only result that was semi-consistent with all of the six linkers was that, if multiple amplified products were produced during amplification (in a single DNA sample), their occurrence was restricted to the younger individuals. Overall both 1-day old, as well as the 4-month and 21-month old samples produced multiple amplimers, while other ages produced a single product.

CHAPTER FOUR: CONCLUSION

We have identified seven biomarkers for human biological age determination. Through triplicate specificity studies, three of these candidate genes COL1A2, HBE1 and IGFBP3, were shown to be expressed at elevated levels in younger aged individuals, newborns, or post-pubertal individuals, respectively. Duplex real-time PCR amplifications were designed and developed for the individual age specific biomarkers with the incorporation of the ribosomal protein, S15 as an internal positive control (IPC) housekeeping gene. Biological age specificity results illustrated that these biomarkers are specific for their target age groups and further analysis will consist of complete Scientific Working Group on DNA Analysis Methods (SWGDAM) validation studies, including sensitivity, species specificity, body-fluid specificity, stability, and the ability of the biomarkers to determine biological age in body-fluid mixtures.

It is estimated that up to 74% of all human genes produce more then one mRNA transcript [119] and given the amount of research that is conducted on the beta-hemoglobin locus regarding the regulation and mechanism of hemoglobin gene switching and gene expression profiling analysis, it is not surprising that new hemoglobin genes are being discovered at an advanced rate. Recently, Goh and colleagues discovered a novel hemoglobin transcript, mu-globin, which aligned with the 3′ region of the pseudo-alpha2 gene on chromosome 16p13.[120] Additionally, this group defined a new delta-globin mRNA (Alt-d), which is formed by an alternate upstream transcription initiation site.[121] This Alt-d transcript exhibited a restricted expression pattern being detected only in fetal liver, bone marrow and adult peripheral blood samples.

In addition to the three biomarkers listed above, we have also identified four novel gamma hemoglobin transcripts (HBG1n1, HBG1n2, HBG2n2 and HBG2n3) that exhibit restricted expression in the blood of (human) newborn children. Individual qRT-PCR assays were developed to measure two of these transcripts in forensic specimens. Adjustment of the primer concentrations in the qRT-PCR reaction permitted the establishment of two temporally delimited assays, one of which was specific to blood from newborns 4-months or under (≤4 months) and the other to newborns who were hours old (<24 hours). Both assays may be useful in a variety of child kidnapping, assault and criminal abortion investigations with the latter (<24 hours) being of particular use for those cases involving hospital abductions.

Validation studies on these qRT-PCR assays revealed that the HBG1n1 and HBG2n3 transcripts appear to be restricted to blood from newborns in the human (or at least, primate) lineage. The assays appear to be robust enough for forensic use, in that the newborn blood-specific transcripts are detectable at least up to 15 months in the dried state. Additionally, the sensitivity of the reactions are compatible with forensic applications, where only a few cell equivalents of total RNA are required (i.e. 50 pg) and >100ng of total RNA is recoverable from typical sized (50-ul) bloodstains [122]. The sensitivity of the assay is thus 50-500 cells assuming 0.1-1.0 pg total RNA per cell [123-125].

Based on our results illustrating that these novel transcripts appear to be created by a rearrangement event occurring within exons two and three of the standard gamma mRNA transcripts (**Figure 42**), the remaining upstream and downstream gene structure would be preserved in all four new transcripts. These four isoforms would include ten of

the twelve nucleotides that form the upstream capping box, as well as the downstream 3'hexanucleotide sequence (AATAAA) found in both gamma-hemoglobin genes and many eukaryotic mRNAs [126, 127]. At the present time the exact mechanism by which these isoforms are created is unknown, but due to the presence of four variable directly repeated sequences in these novel genes, a direct repeat mediated alternative splicing mechanism is theorized. Secondary structure analysis of the standard hemoglobin genes revealed the presence of a centralized multi-loop surrounded by branching stem loop structures. Additionally, determining if these highly regulated transcripts play a role in the functional development of an organism is yet to be determined.

The location of the recombination events raised an additional question as to the ability of these newborn transcripts to encode proteins. Inputting the newly formed mRNA sequences into EditSeq translation software from DNAstar LaserGene, revealed that all four mRNAs contained an open reading frame (ORF), and hence contained the ability to encode a protein, even with partially deleted exons (**Figure 43**). It should be noted that the statistical probability of all four transcripts, each comprised of a specific and individual break-point sequence, forming an ORF is ~0.012 or $(1/3)^4$. The conserved portions for mRNA translation and protein synthesis included an initiation start codon present in exon one, along with three new alternate stop codons formed by the HBG1n1, HBG1n2, and HBG2n3 sequences and one conserved stop codon, within the HBG2n2 deleted sequence. Analysis of the alternate protein sequences in **Figure 43** illustrated that the HBG1n1 and HBG1n2 sequences aligned with the standard HBG1 protein from AA1-80 and AA1-75, respectively, and both had the entire C-terminal region of the HBG1 protein deleted. The recombination region of the HBG1n2 transcript brings its reading

frame a single nucleotide in front of the termination codon of the HBG1 sequence and due to its alternate reading frame, the ORF continues into the 3' UTR of the HBG1 sequence. In the HBG1n1 isoform the downstream pentanucleotide recombining region occurs in the 3' UTR of exon 3, and the encoded protein has a similar C-terminal sequence to the HBG1n2 isoform. Analysis of the G-gamma isoforms revealed that HBG2n2 aligns with the HBG2 sequence in the N-terminal region from amino acids 1-86, and rejoins in the C-terminal region encoding the final six amino acids of the hemoglobin proteins. In contrast to the other isoforms, the HBG2n3 sequence creates a stop codon immediately after the recombination event, to generate a truncated protein of only 92 amino acids. At the present time, it is unknown whether proteins are actually being produced from these novel transcripts, however further analysis of these isoforms will consist of an immunoassay for the detection of the potentially encoded truncated proteins, in addition to transcript verification in newborn blood and select fetal tissues, by RNA specific northern blot examination.

We have reported on the detection of seven mRNA transcripts whose expression levels are increased during specific developmental stages of the human lifecycle. Forensic real-time PCR assays have been designed, developed and optimized for facile sample analysis and biological age determination of newborns, younger aged individuals and post-pubertal populations. These assays therefore provide investigators additional probative information, in addition to the genetic profile of an individual, concerning the physical characteristic of biological age.

APPENDIX A: FIGURES

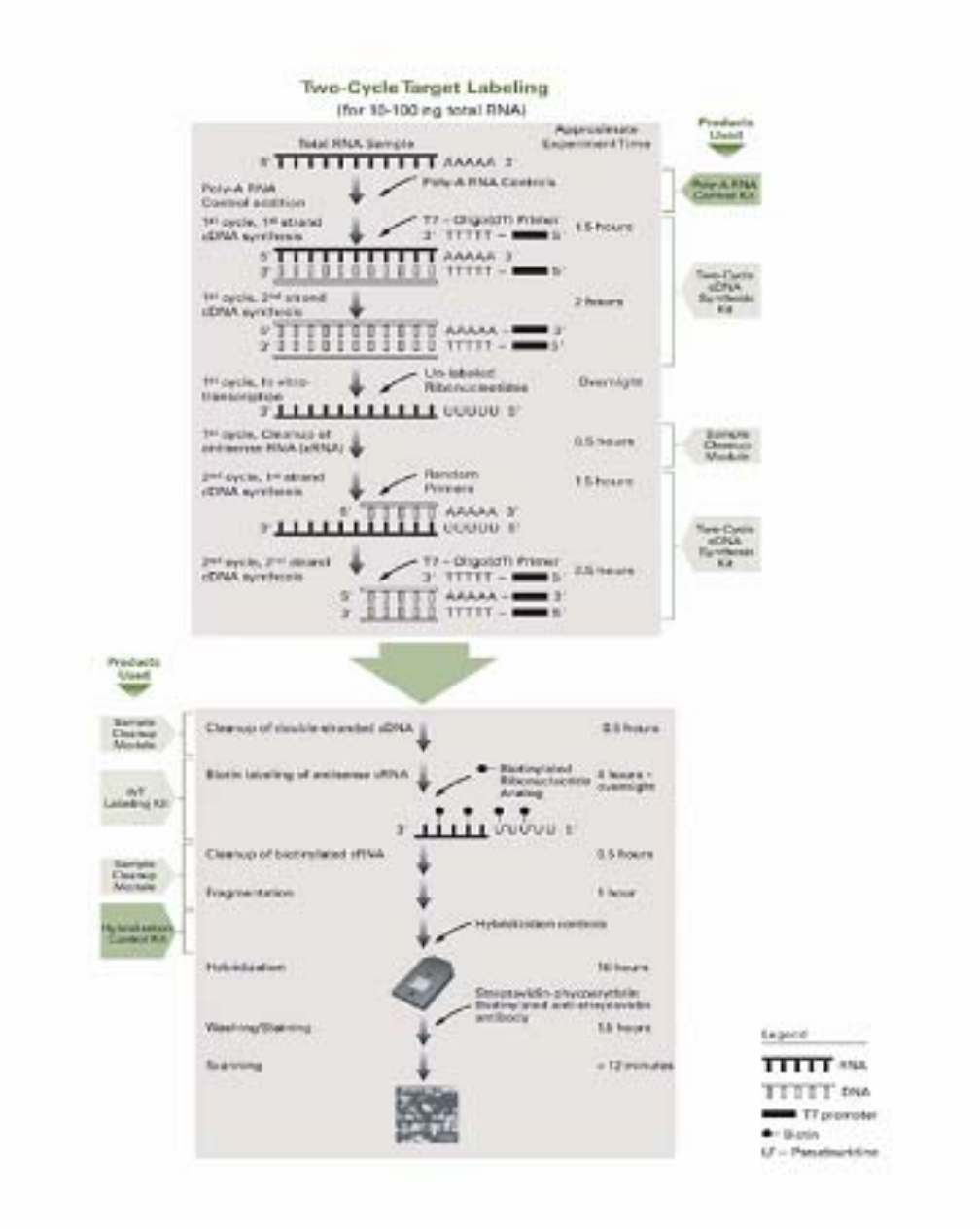
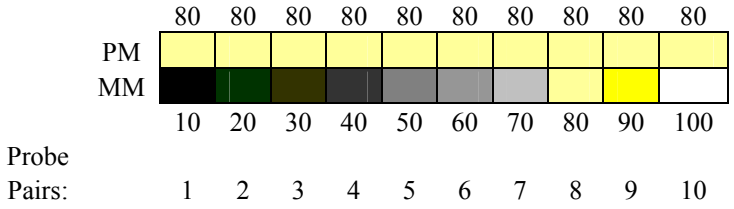


Figure 1: Two-Cycle cRNA Preparation Scheme for AFFYMETRIX GeneChip Hybridization.

$$R = (PM - MM) / (PM + MM)$$



Probe Pairs	PM score	MM score	R value
1	80	10	0.7778
2	80	20	0.6000
3	80	30	0.4545
4	80	40	0.3333
5	80	50	0.2308
6	80	60	0.1429
7	80	70	0.0667
8	80	80	0.0000
9	80	90	-0.0588
10	80	100	-0.1111

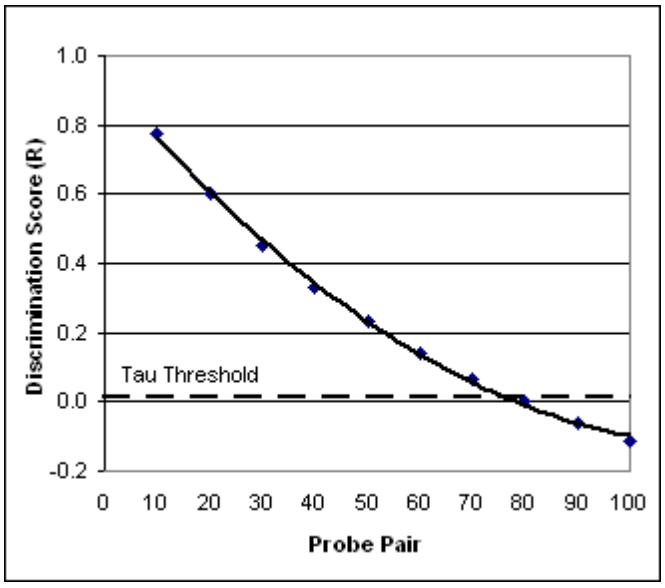


Figure 2: Discrimination Score Calculation for a Hypothetical Probe Set.

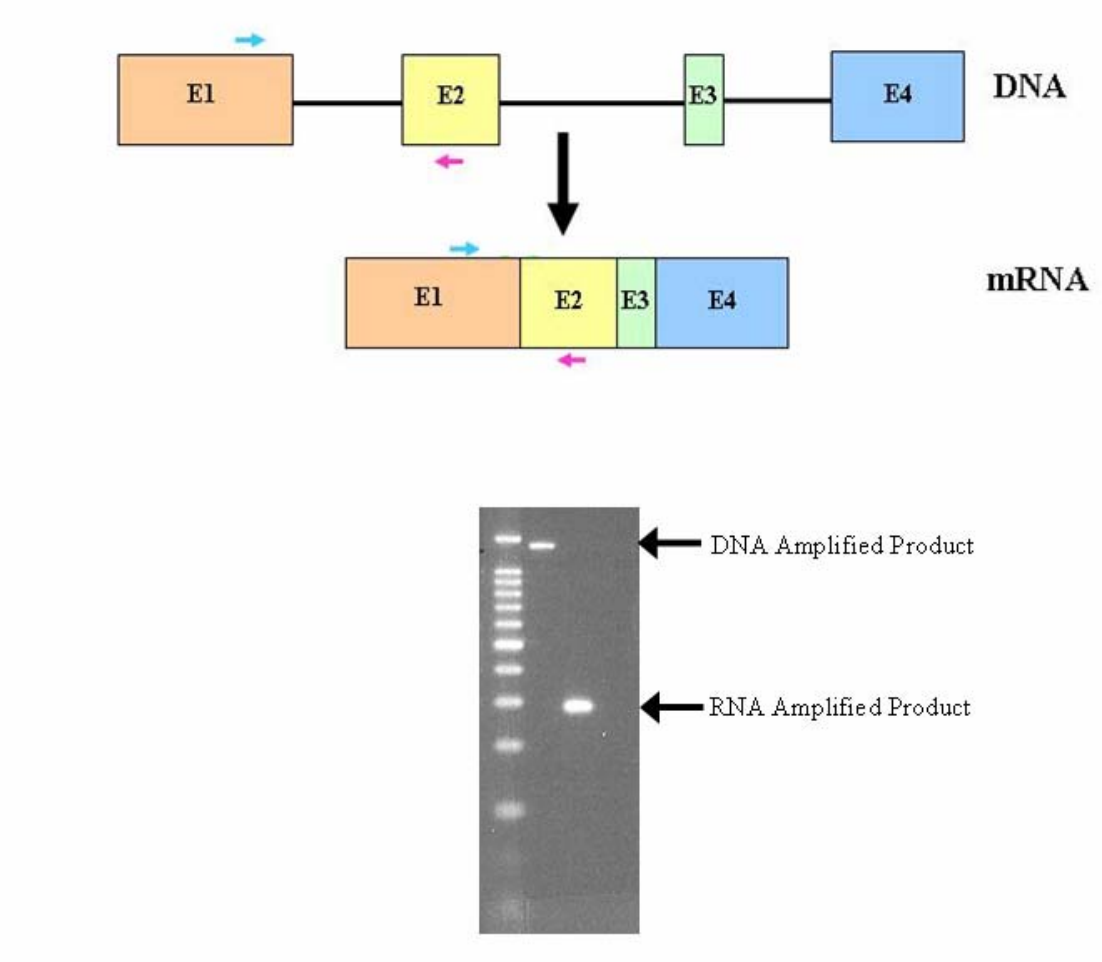


Figure 3: RT-PCR Primer Design.

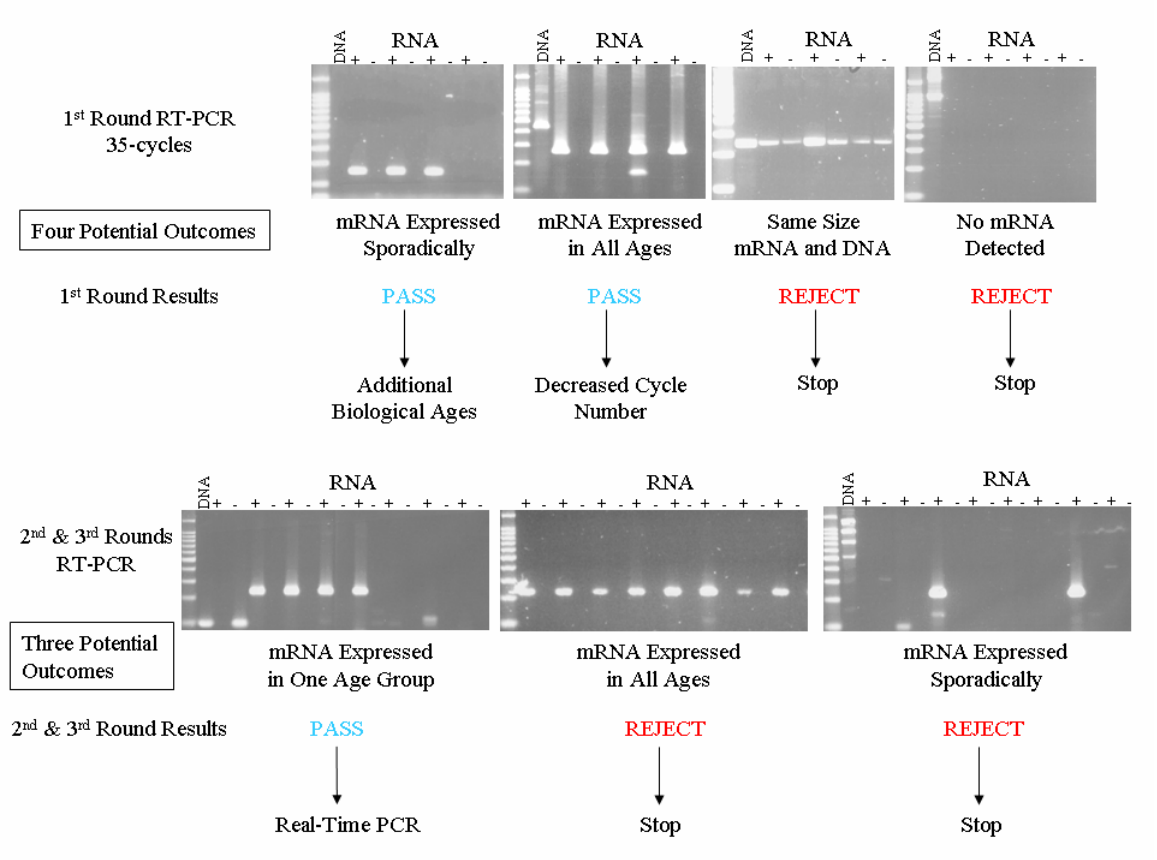
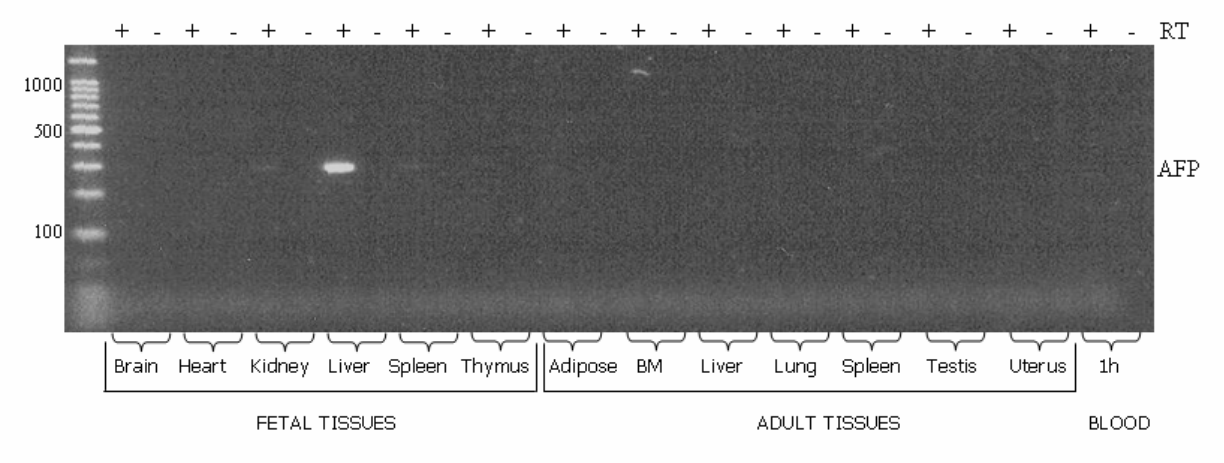
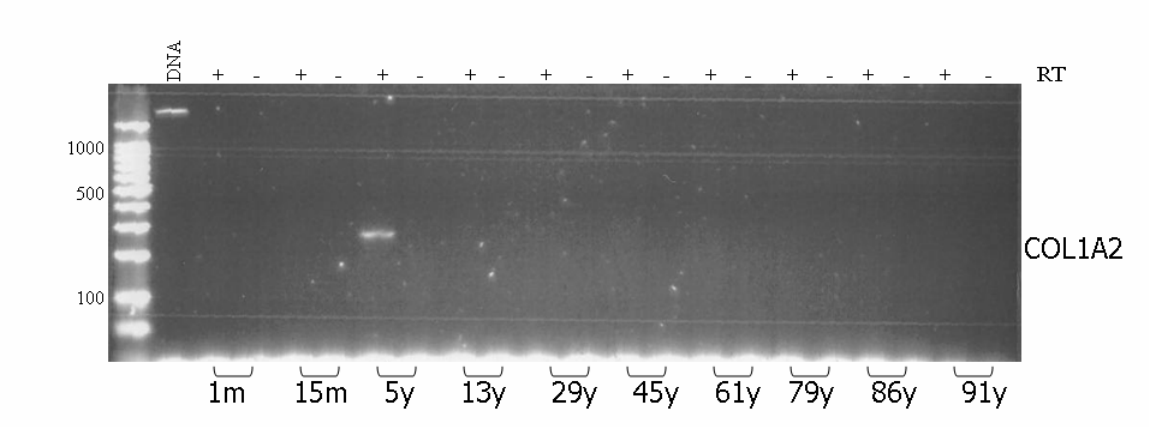


Figure 4: RT-PCR Procedure for Candidate Gene Testing.


+ - + - + - + - + - + - + - + - + - + - + - + - + - + - RT
1000
500
100
AFP
Brain Heart Kidney Liver Spleen Thymus
Adipose BM Liver Lung Spleen Testis Uterus
1h
FETAL TISSUES
ADULT TISSUES
BLOOD


DNA
+ - + - + - + - + - + - + - + - + - + - + - RT
1000
500
100
COL1A2
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y

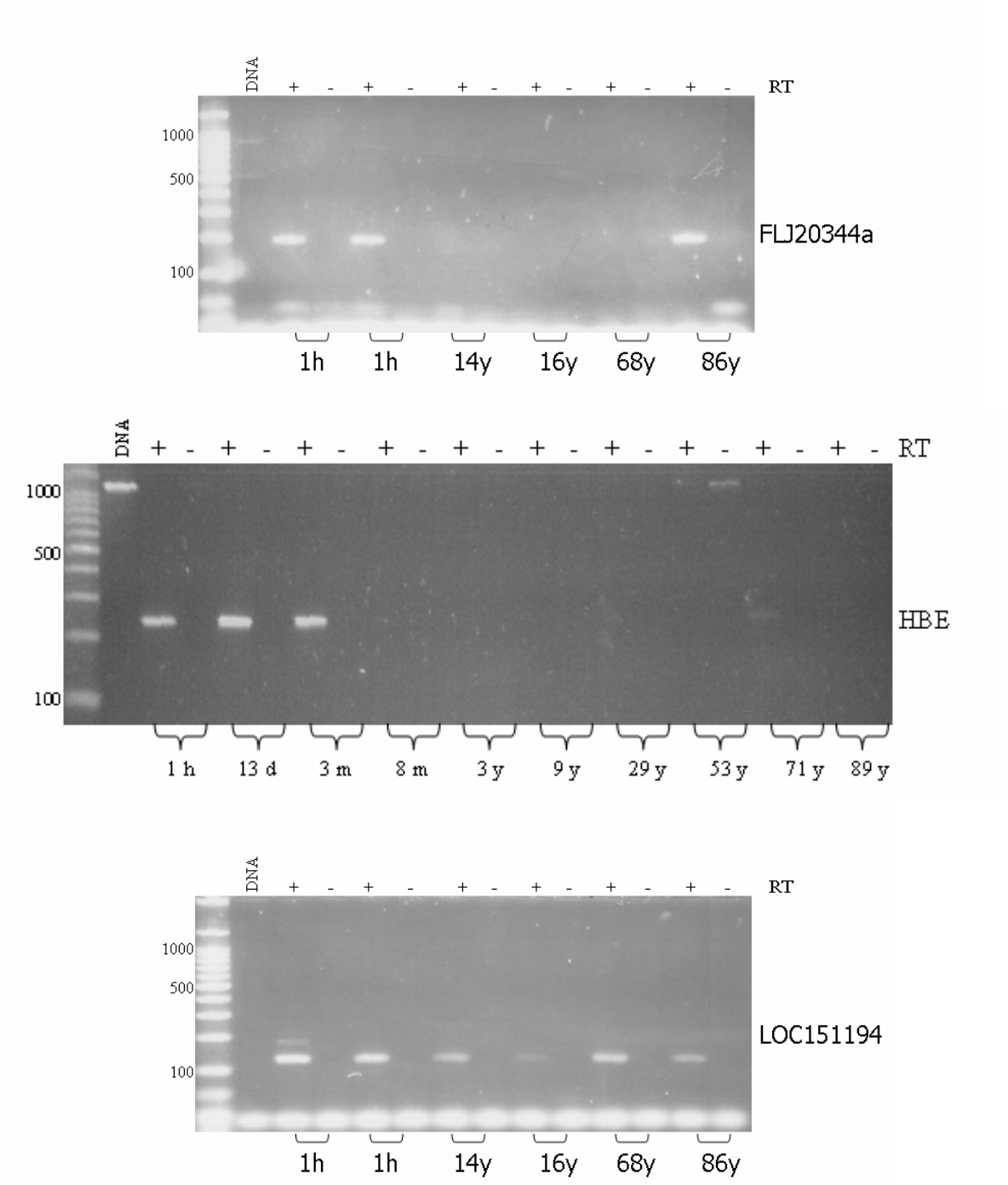
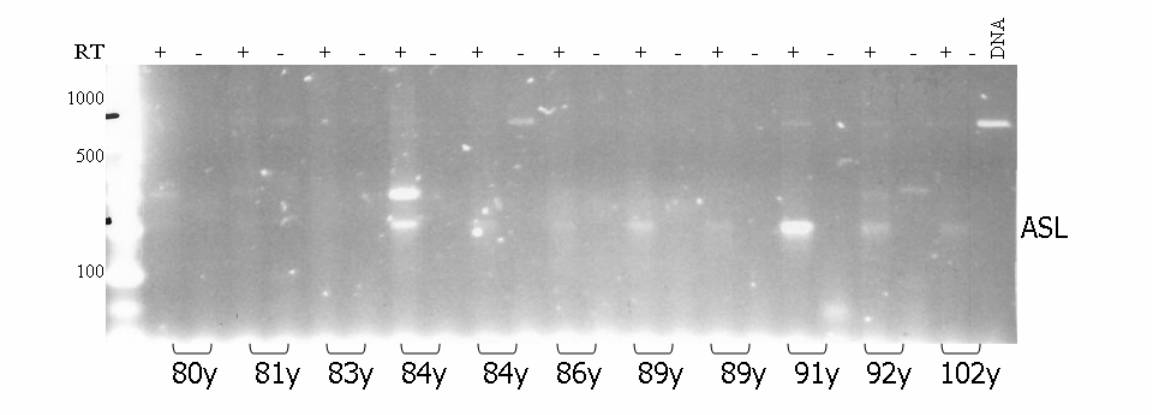
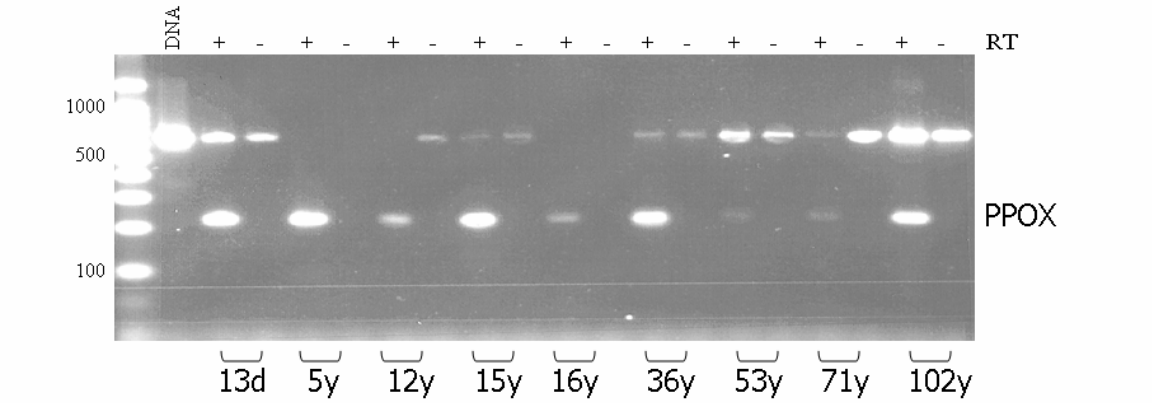
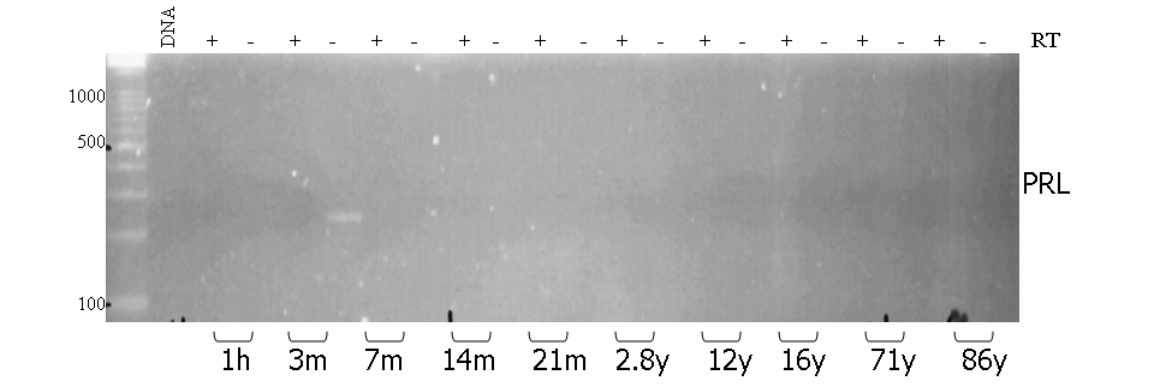
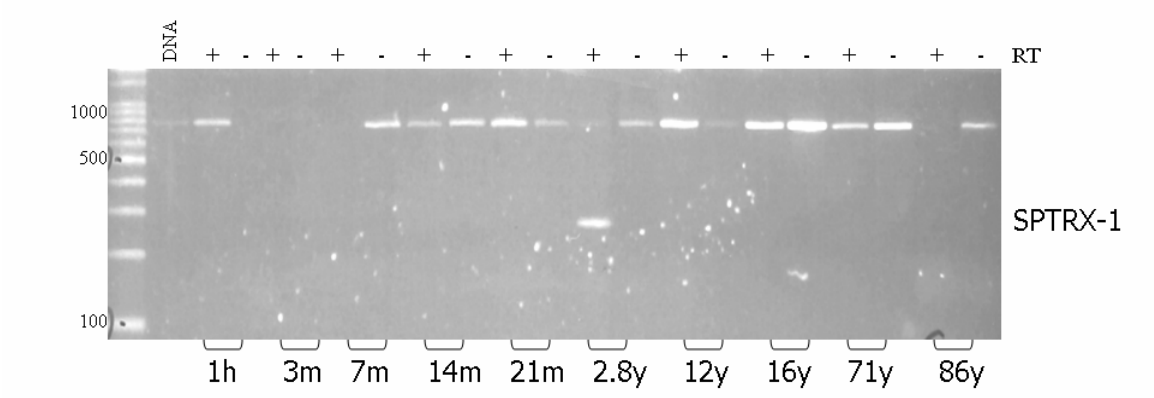


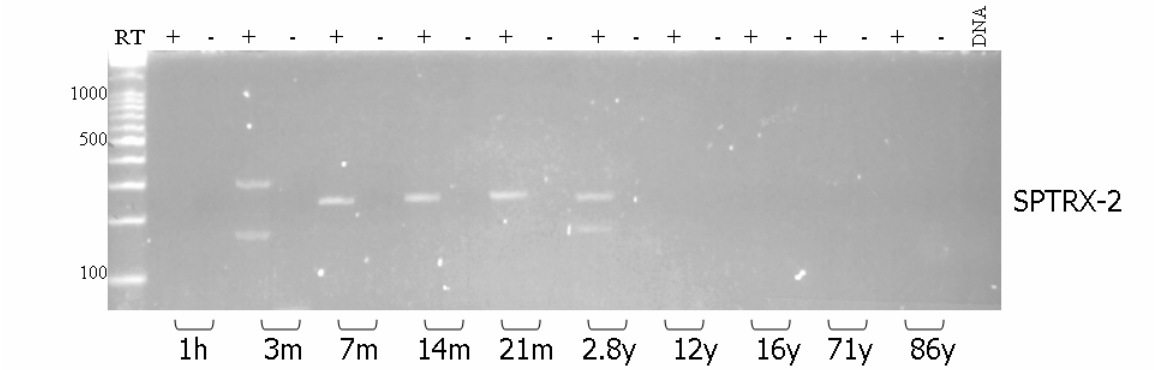
Figure 5: RT-PCR Newborn Candidates Taken to Real-Time PCR.

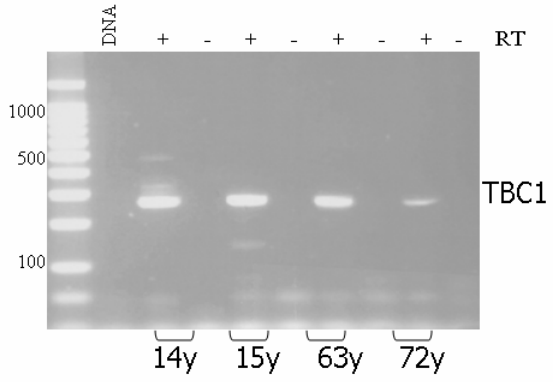

RT
DNA
1000
500
100
ASL
80y
81y
83y
84y
84y
86y
89y
89y
91y
92y
102y


DNA
RT
1000
500
100
PPOX
13d
5y
12y
15y
16y
36y
53y
71y
102y


DNA
RT
1000
500
100
PRL
1h
3m
7m
14m
21m
2.8y
12y
16y
71y
86y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
SPTRX-1
1h 3m 7m 14m 21m 2.8y 12y 16y 71y 86y


RT + - + - + - + - + - + - + - + - + - + - DNA
1000
500
100
SPTRX-2
1h 3m 7m 14m 21m 2.8y 12y 16y 71y 86y


DNA
+ - + - + - + - RT
1000
500
100
TBC1
14y 15y 63y 72y

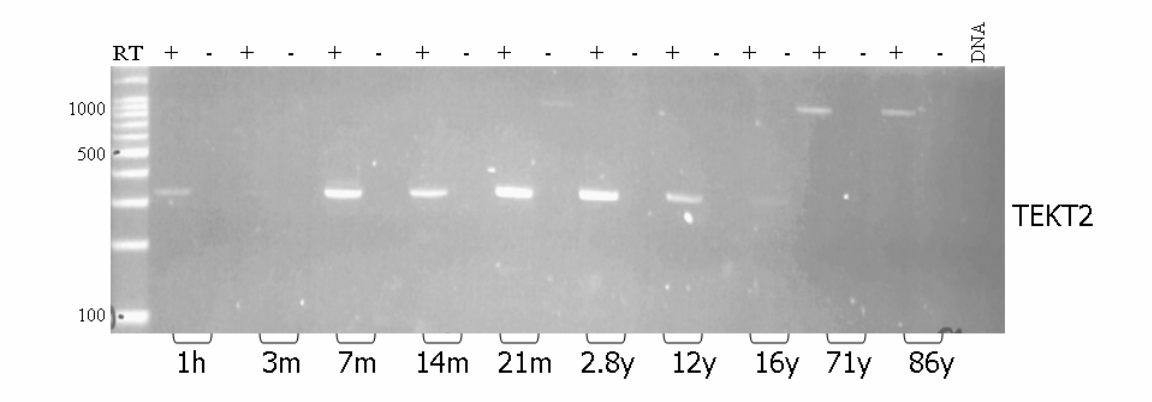
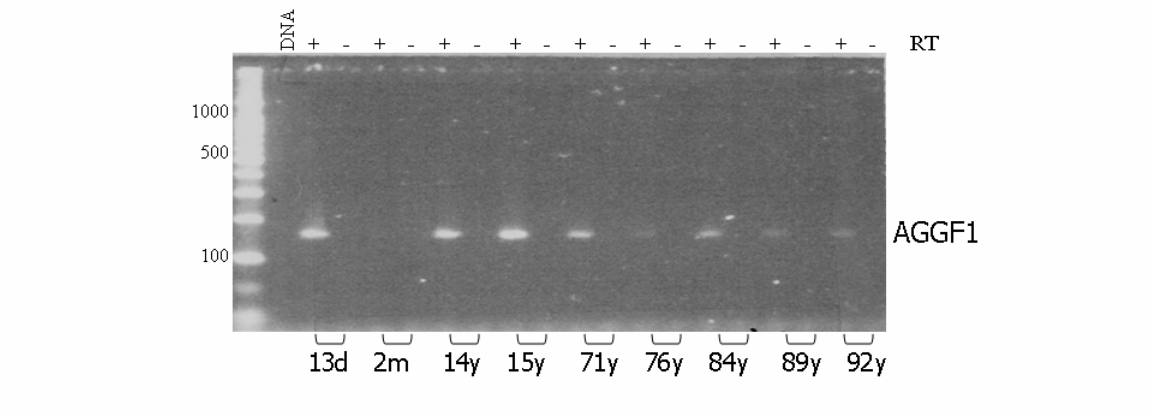
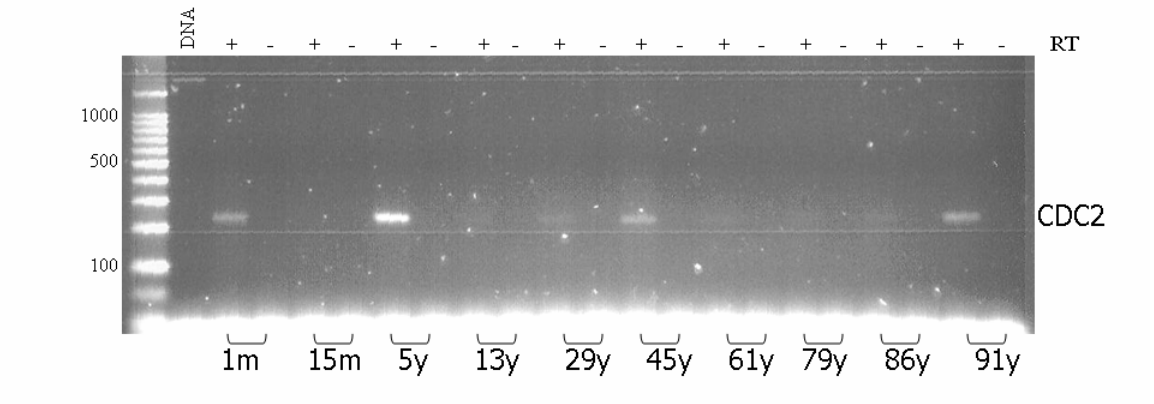
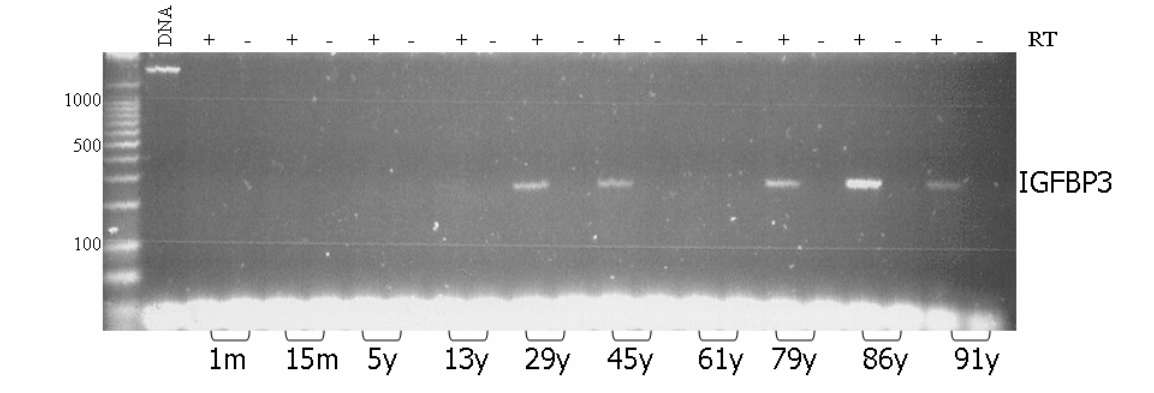
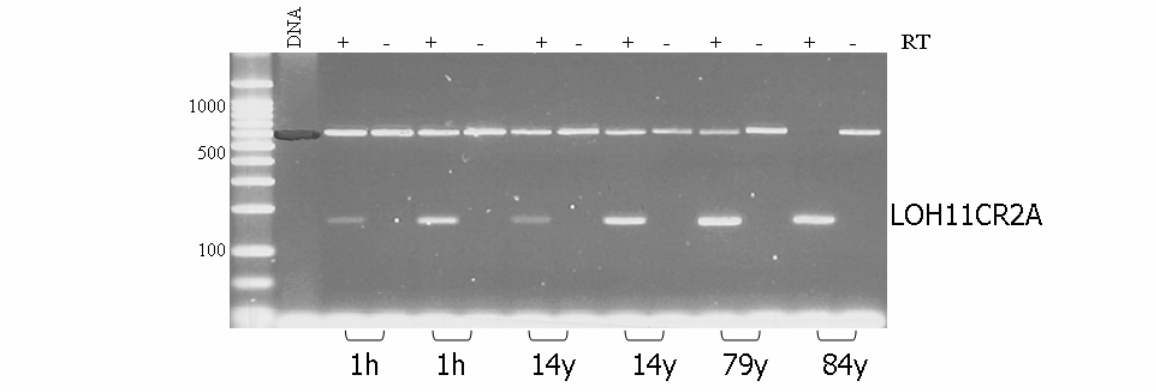


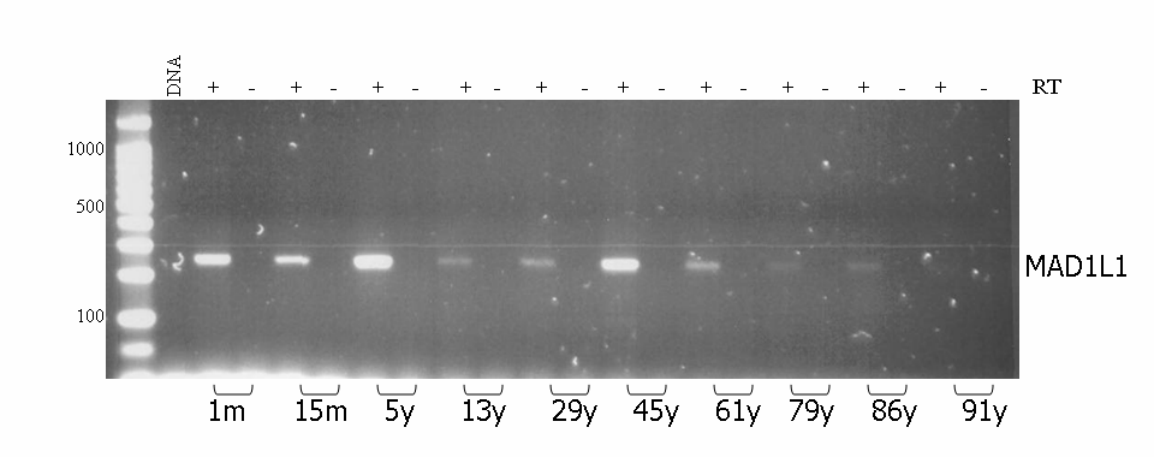
Figure 6: RT-PCR Juvenile Candidates Taken to Real-Time PCR.

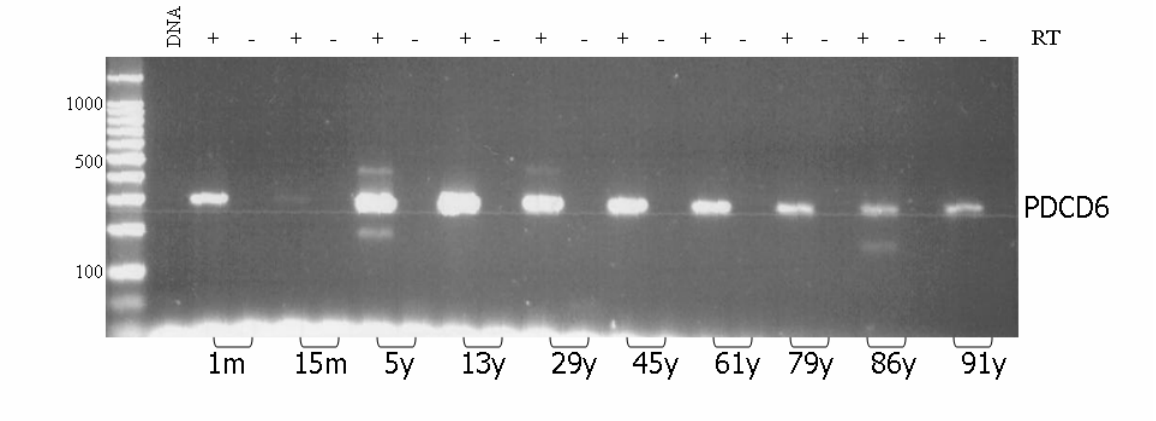

DNA
+ - + - + - + - + - + - + - + - + -
RT
1000
500
100
AGGF1
13d 2m 14y 15y 71y 76y 84y 89y 92y

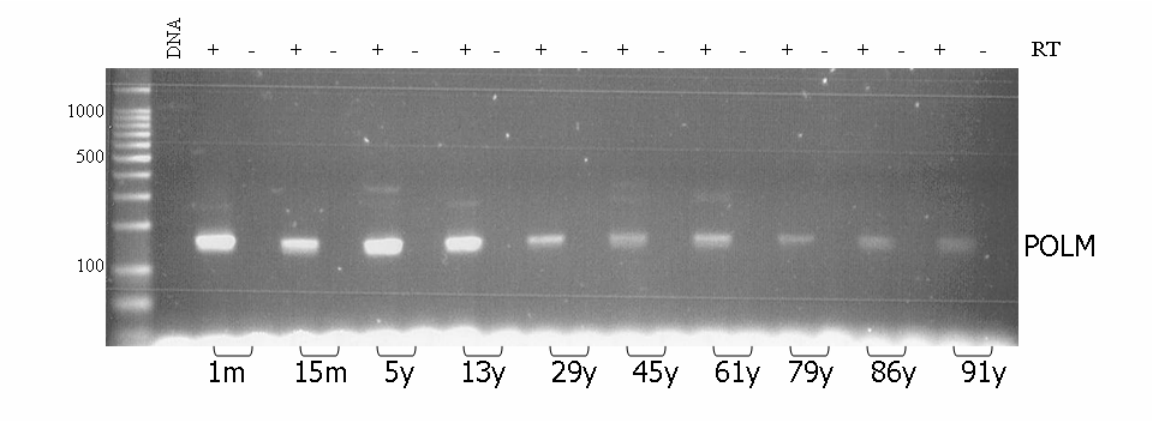

DNA
+ - + - + - + - + - + - + - + - + - + -
RT
1000
500
100
CDC2
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y

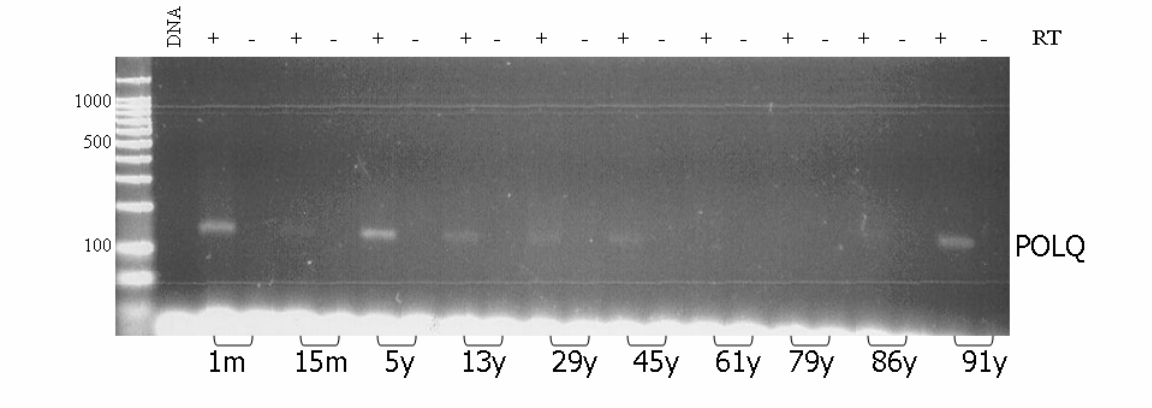

DNA
+ - + - + - + - + - + - + - + - + - + -
RT
1000
500
100
IGFBP3
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y

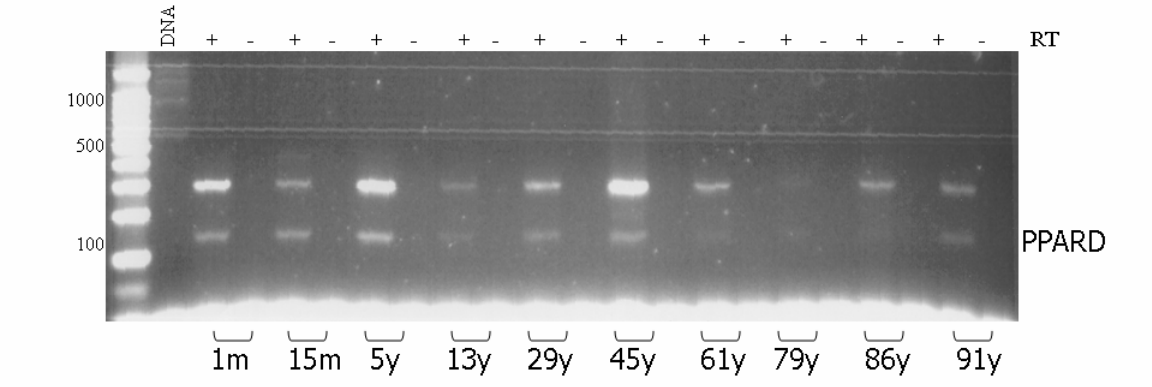

DNA
+ - + - + - + - + - + - RT
1000
500
100
LOH11CR2A
1h 1h 14y 14y 79y 84y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
MAD1L1
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
PDCD6
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y


DNA
+ - + - + - + - + - + - + - + - + - + -
RT
1000
500
100
POLM
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y


DNA
+ - + - + - + - + - + - + - + - + - + -
RT
1000
500
100
POLQ
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y


DNA
+ - + - + - + - + - + - + - + - + - + -
RT
1000
500
100
PPARD
1m 15m 5y 13y 29y 45y 61y 79y 86y 91y

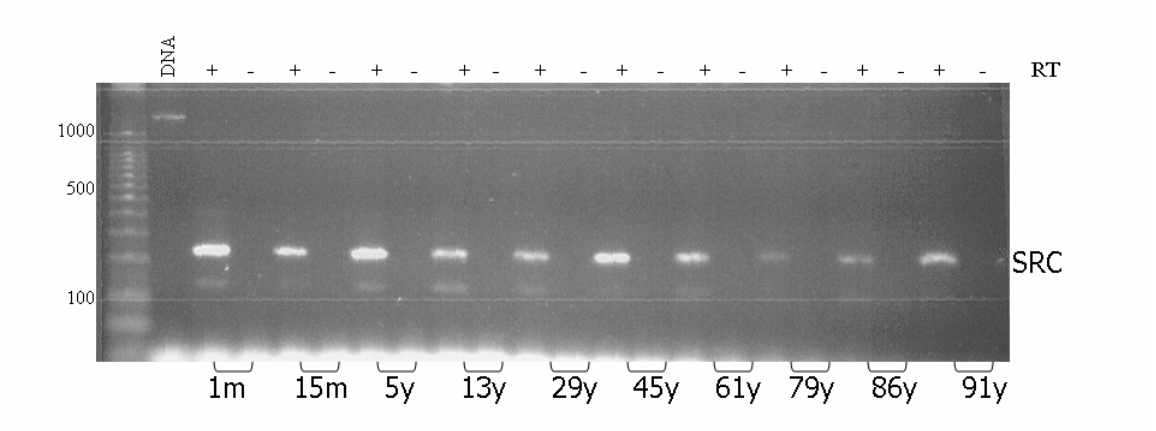
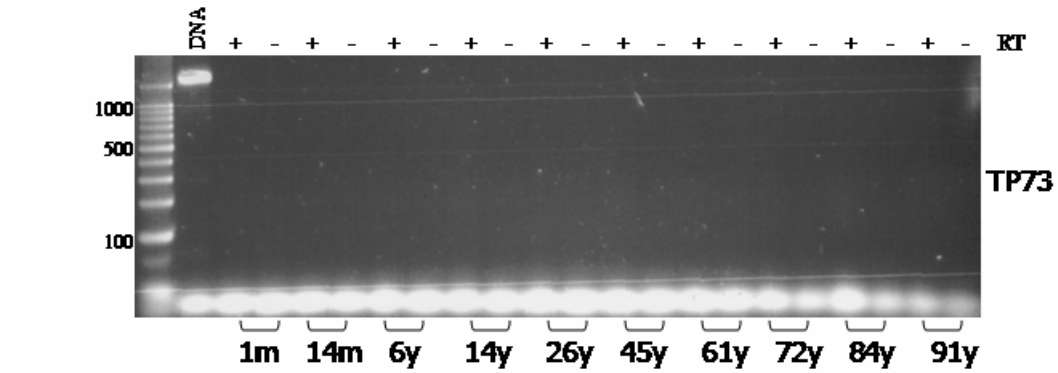
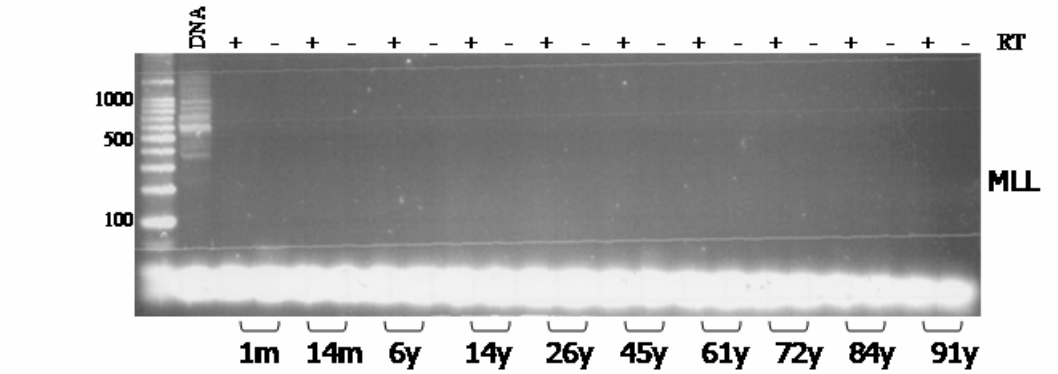
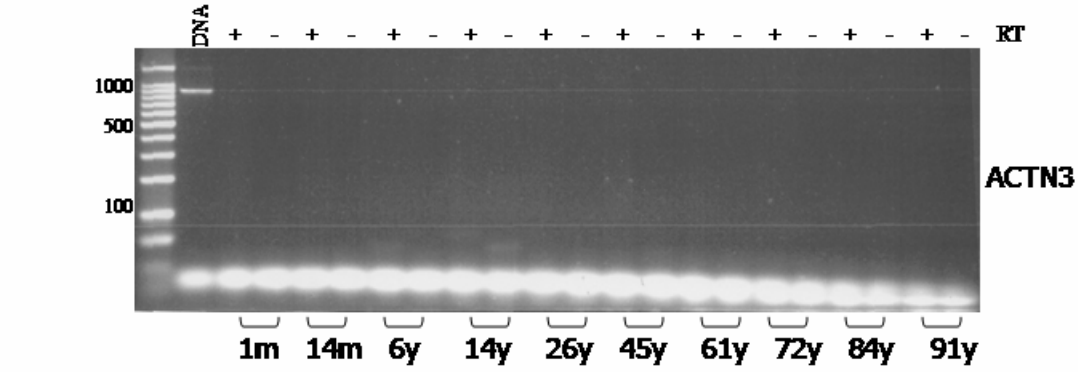
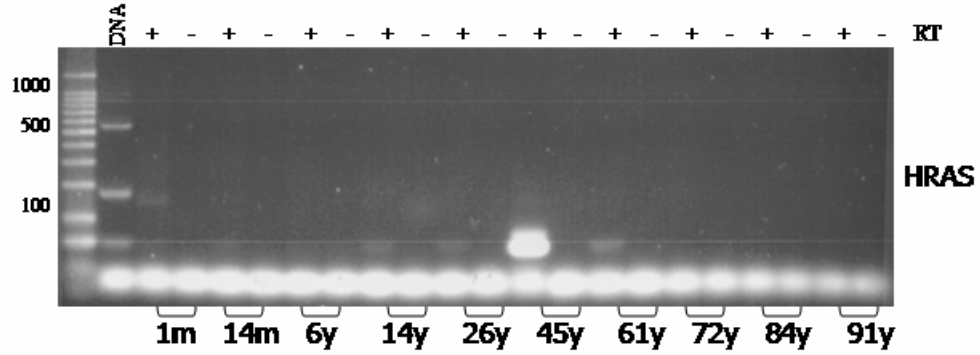


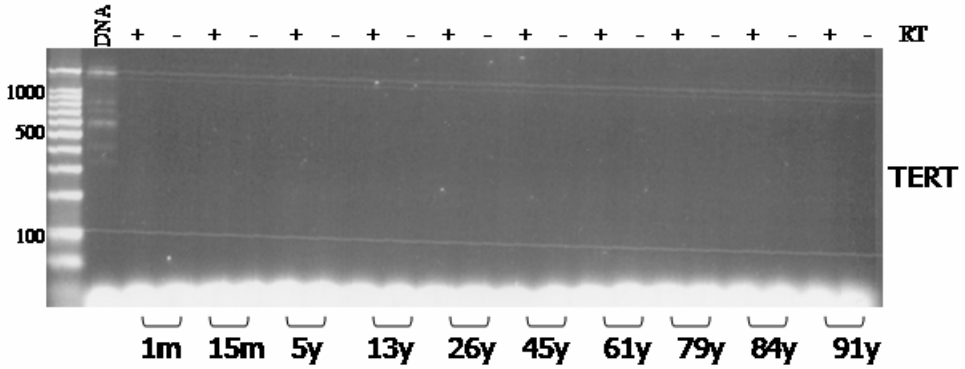
Figure 7: RT-PCR Elderly Candidates Taken to Real-Time PCR.

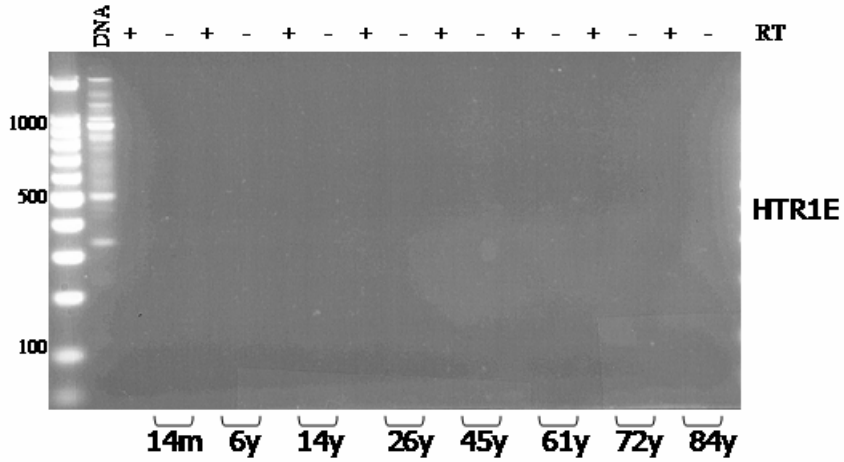

DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
TP73
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
MLL
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
ACTN3
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + -
RT
1000
500
100
HRAS
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + -
RT
1000
500
100
TERT
1m 15m 5y 13y 26y 45y 61y 79y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + -
RT
1000
500
100
HTR1E
14m 6y 14y 26y 45y 61y 72y 84y

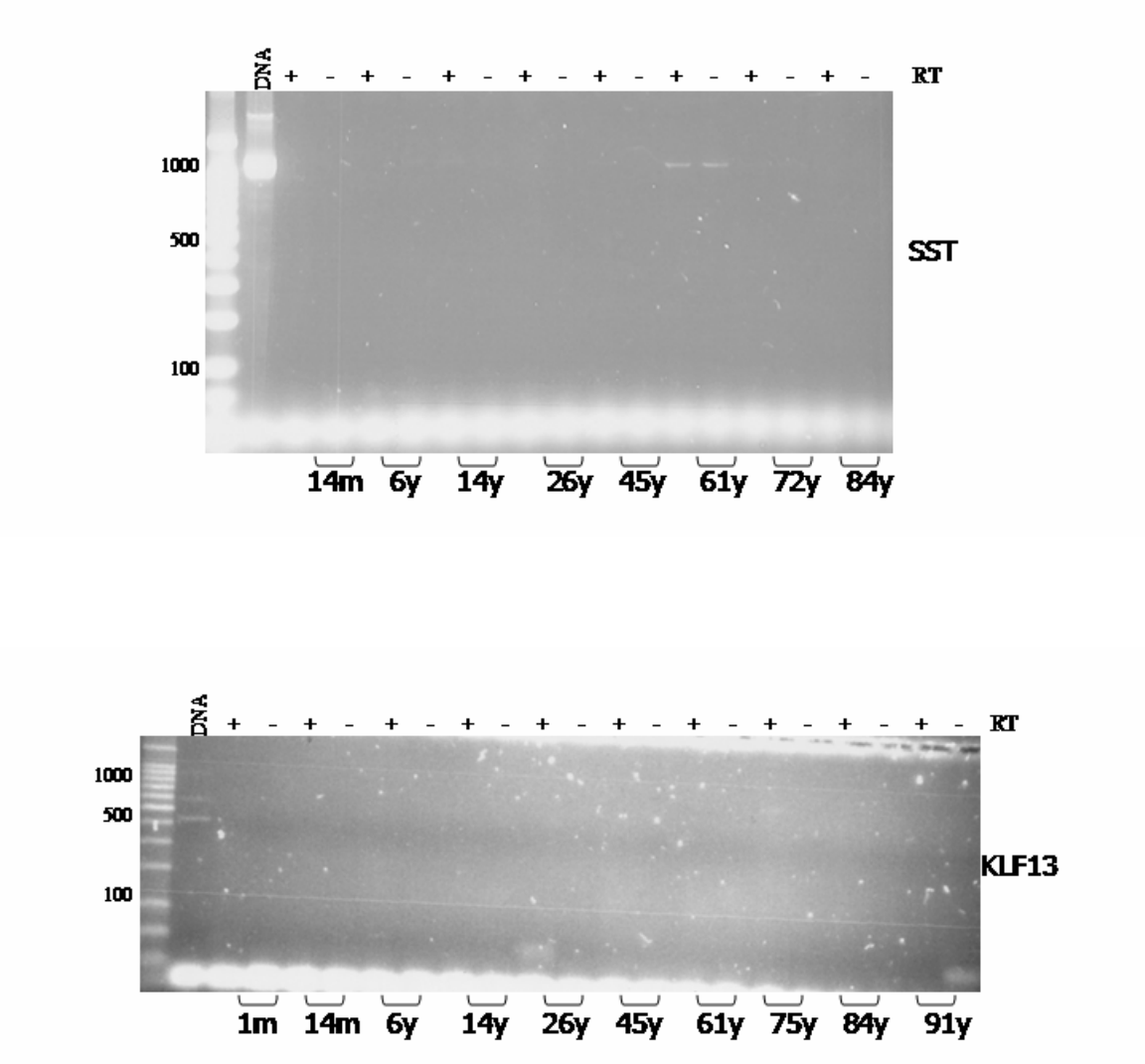
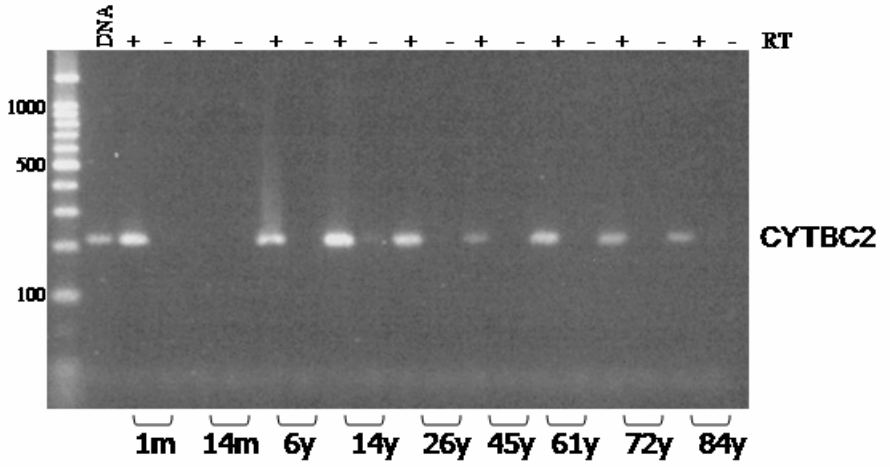
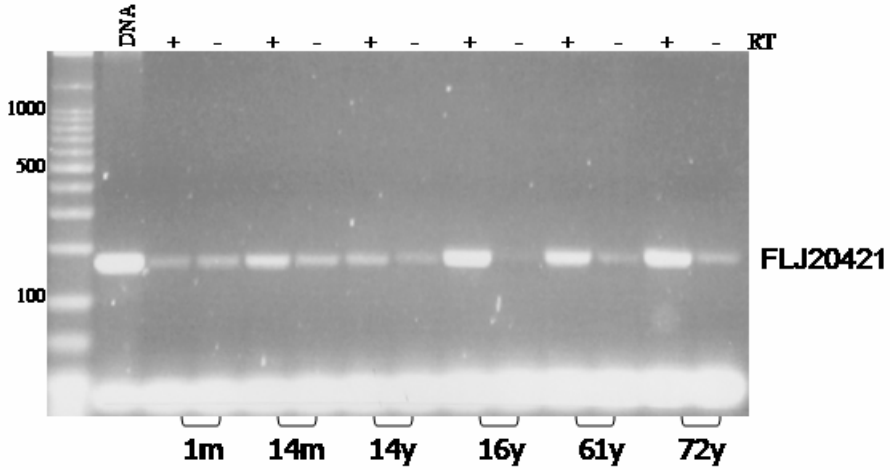
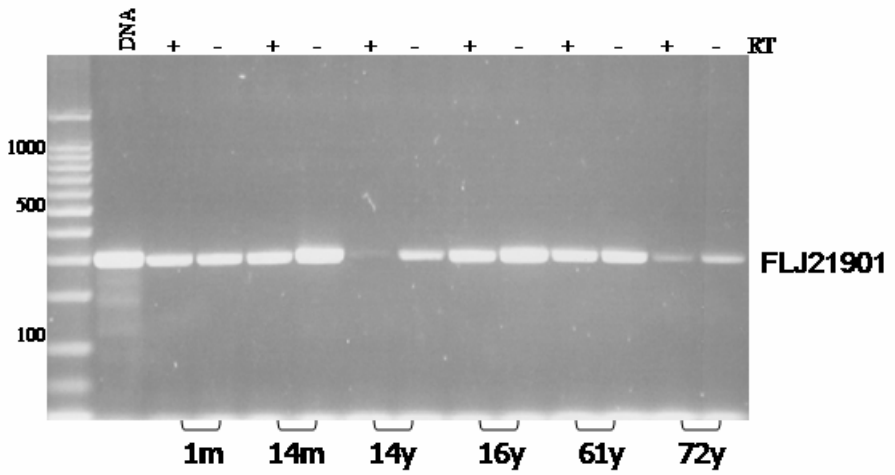
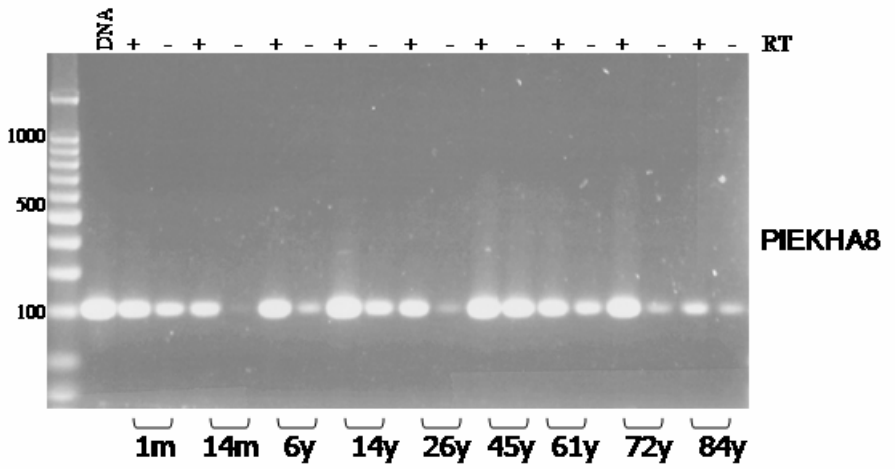


Figure 8: RT-PCR No mRNA/cDNA Product Rejected Candidates.


DNA
+ - + - + - + - + - + - + - + - + - RT
1000
500
100
CYTBC2
1m 14m 6y 14y 26y 45y 61y 72y 84y


DNA
+ - + - + - + - + - + - RT
1000
500
100
FLJ20421
1m 14m 14y 16y 61y 72y


DNA
+ - + - + - + - + - + - RT
1000
500
100
FLJ21901
1m 14m 14y 16y 61y 72y


DNA
+ - + - + - + - + - + - + - + - + - RT
1000
500
100
PIEKHA8
1m 14m 6y 14y 26y 45y 61y 72y 84y

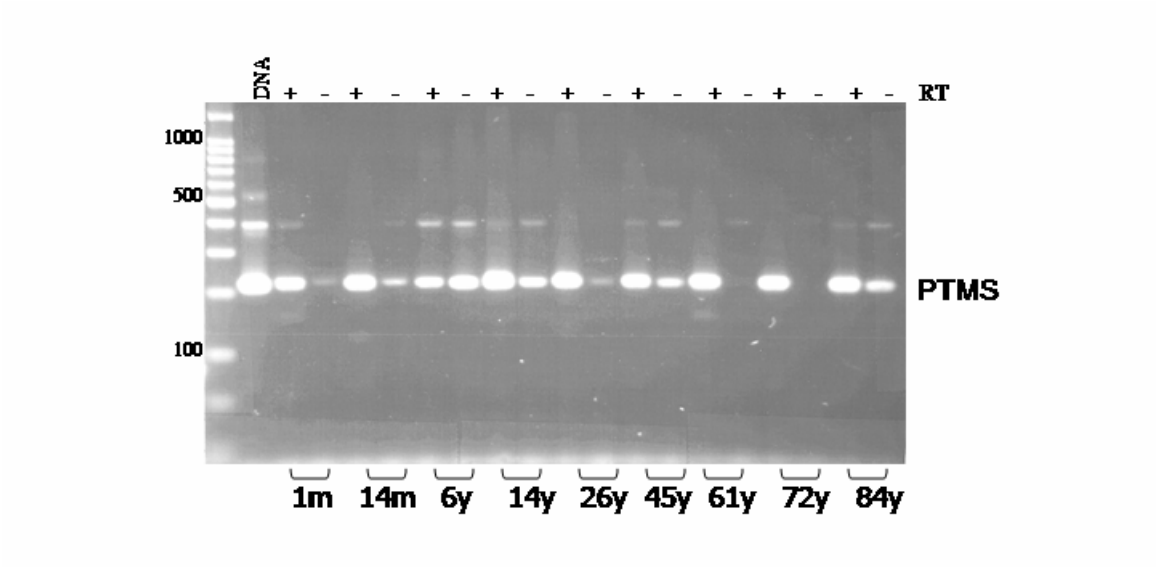
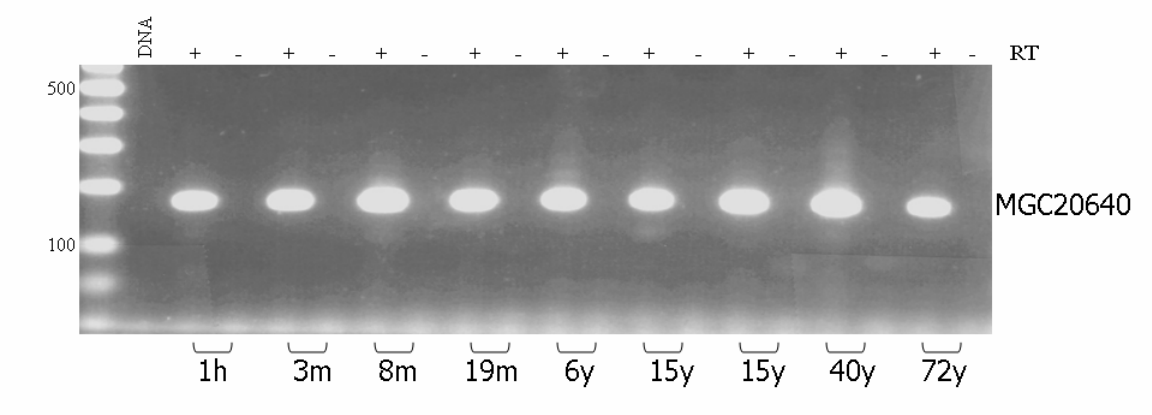
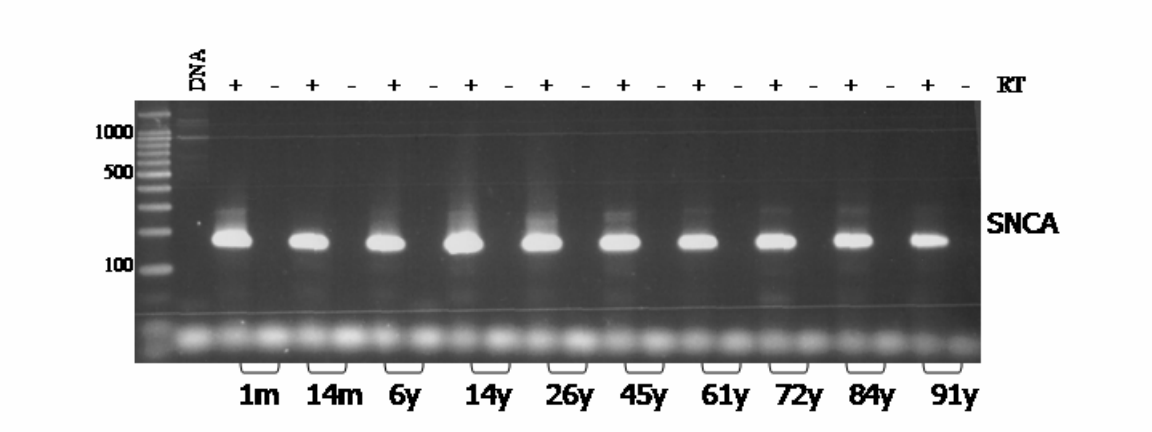
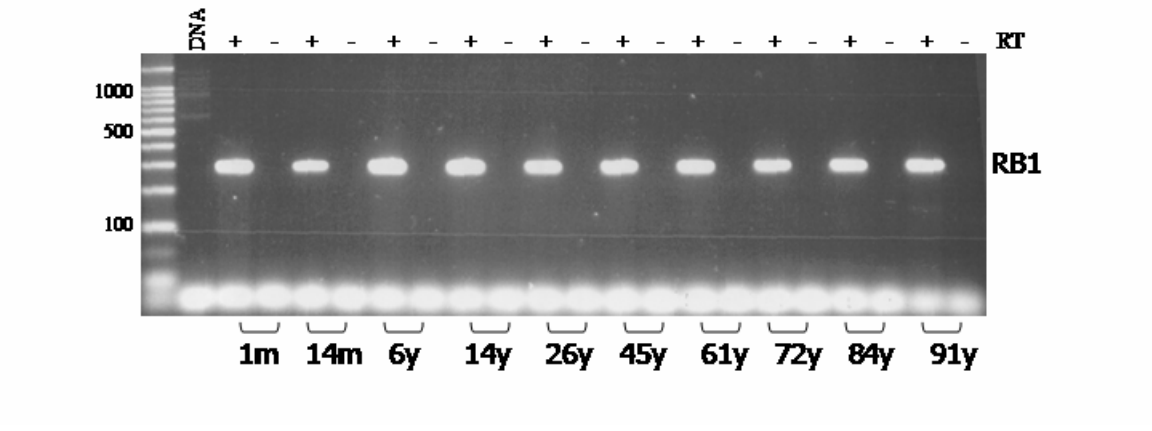
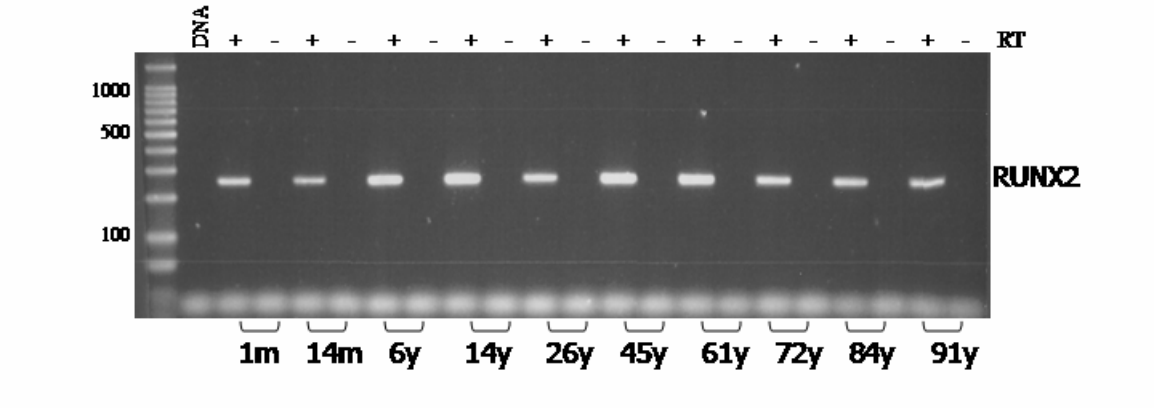


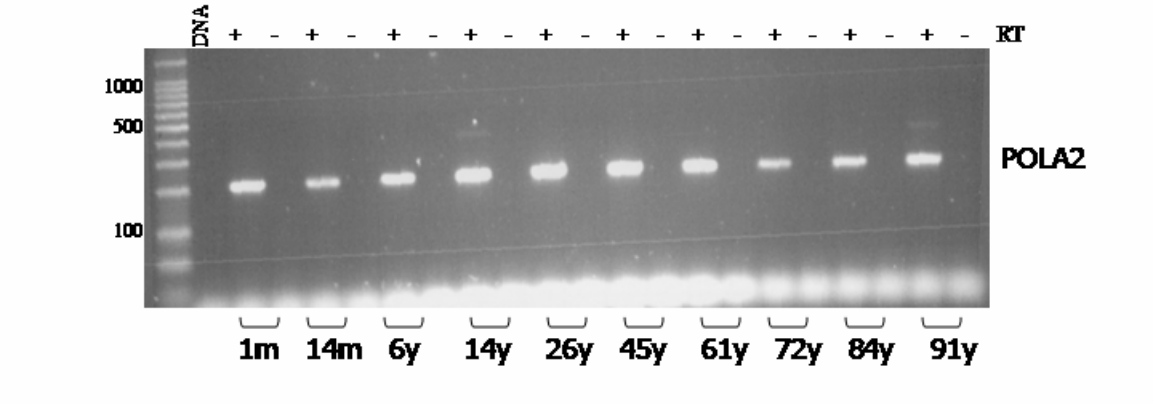
Figure 9: RT-PCR Same Size mRNA/cDNA and Genomic DNA Rejected Candidates

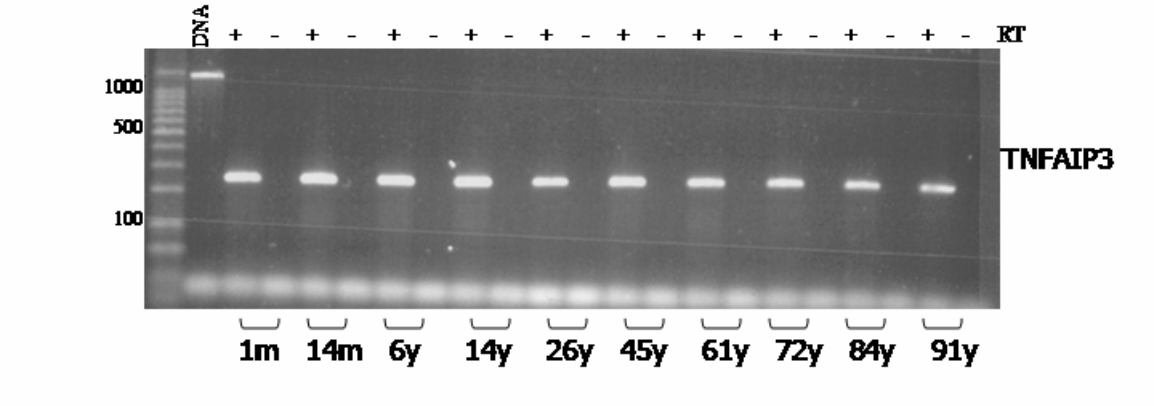

DNA
+ - + - + - + - + - + - + - + - + - RT
500
100
MGC20640
1h 3m 8m 19m 6y 15y 15y 40y 72y


DNA
+ - + - + - + - + - + - + - + - + - RT
1000
500
100
SNCA
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - RT
1000
500
100
RB1
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
RUNX2
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
POLA2
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
TNFAIP3
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y

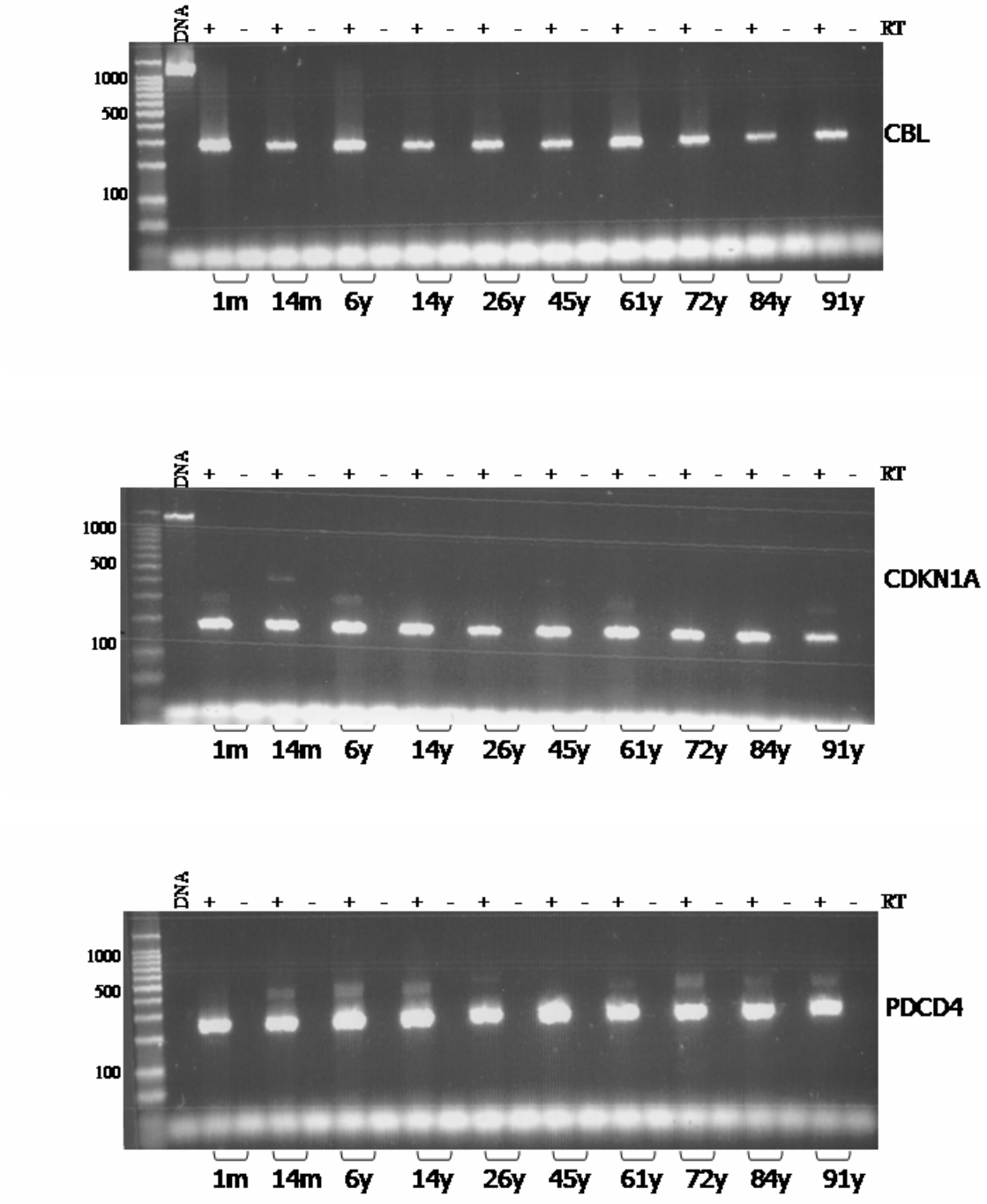
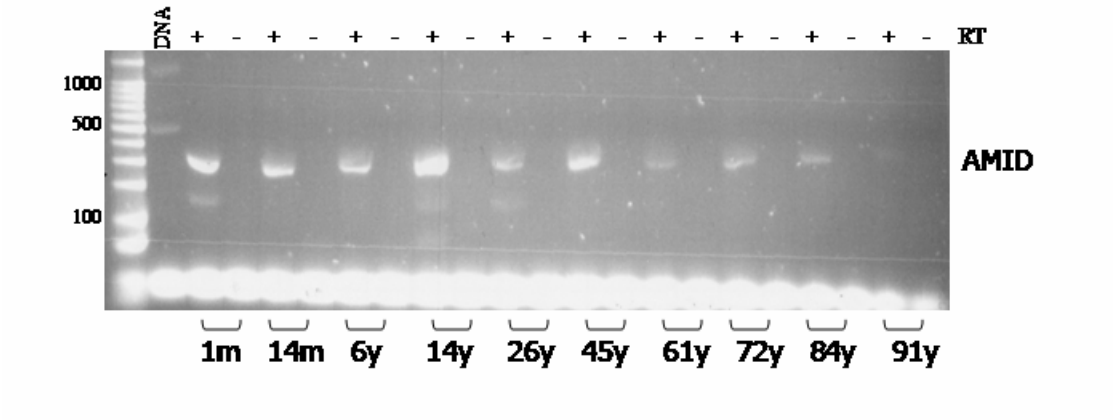
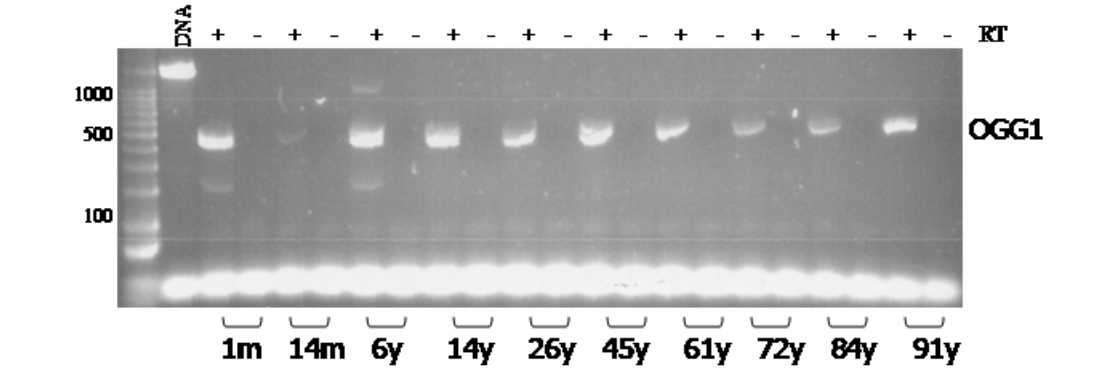
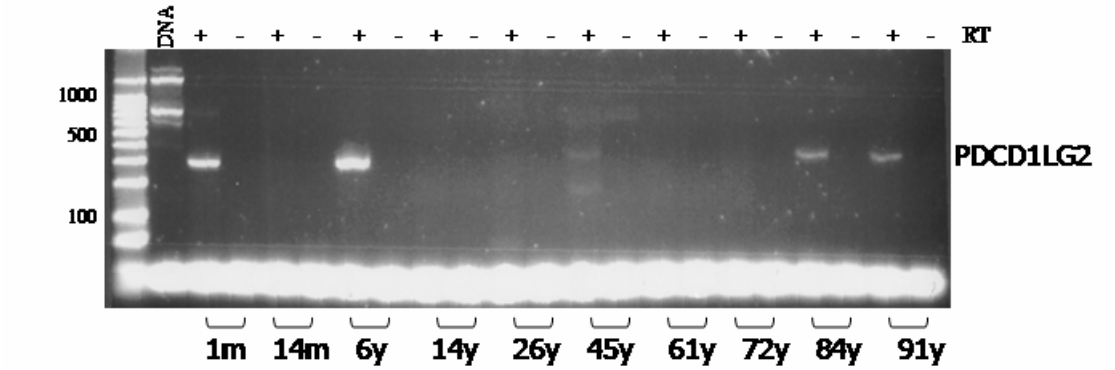
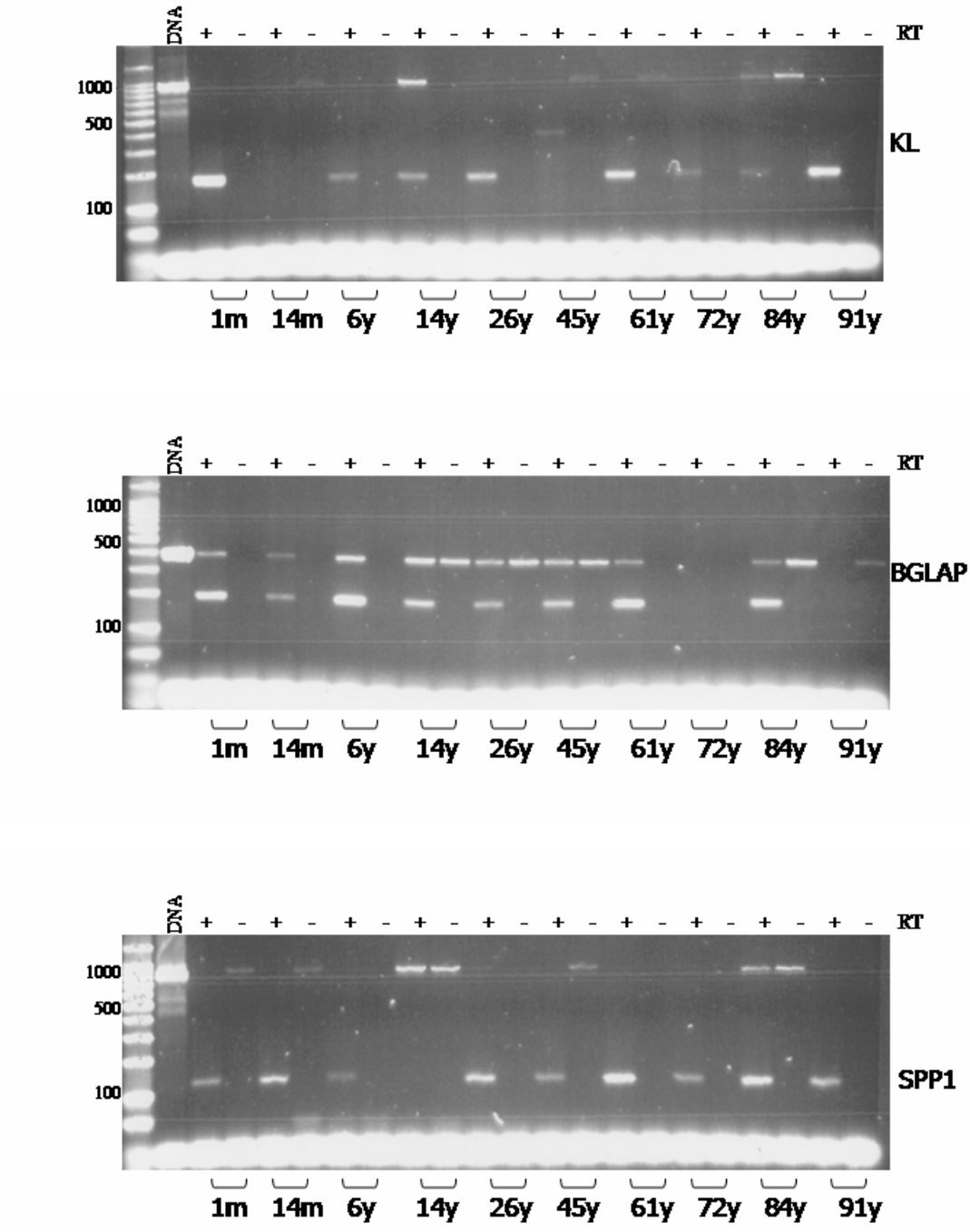


Figure 10: RT-PCR mRNA/cDNA Expression in all Biological Ages Rejected Candidates.


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
AMID
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
OGG1
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
PDCD1LG2
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y


DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
KL
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y
DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
BGLAP
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y
DNA
+ - + - + - + - + - + - + - + - + - + - RT
1000
500
100
SPP1
1m 14m 6y 14y 26y 45y 61y 72y 84y 91y

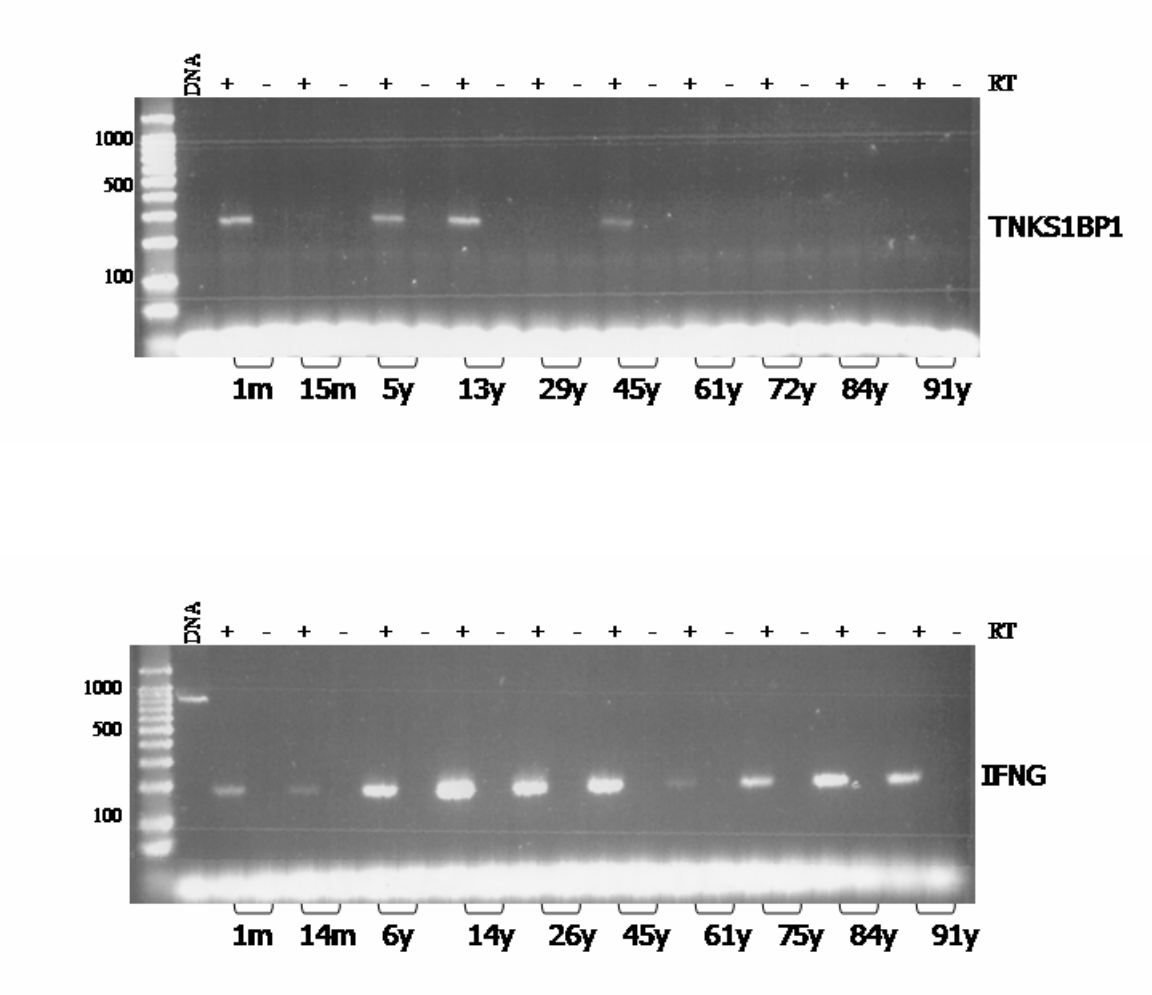


Figure 11: RT-PCR Sporadic mRNA/cDNA Expression in Biological Ages Rejected Candidates.

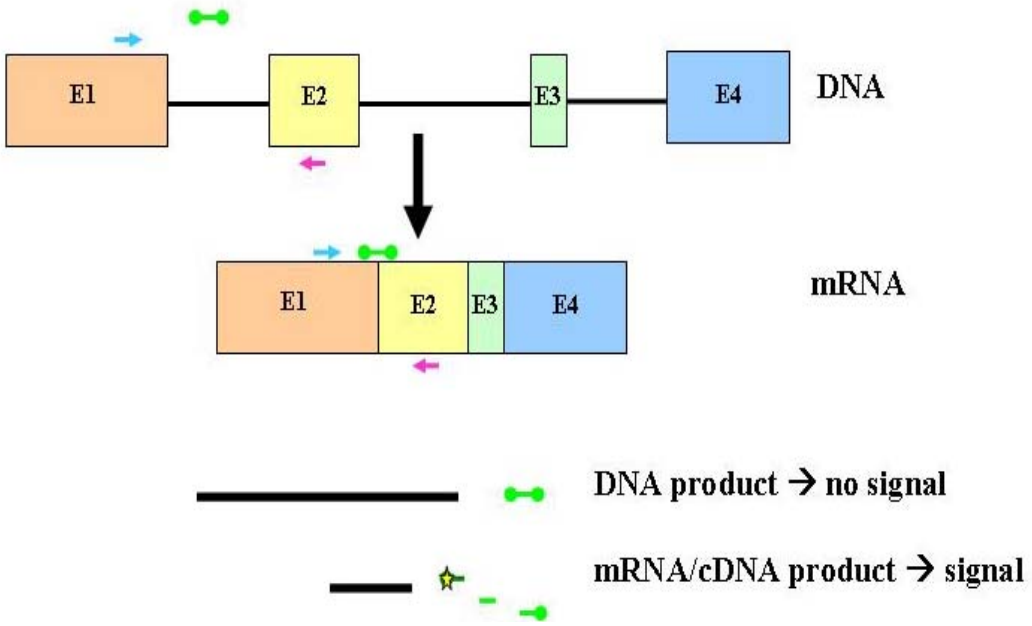


Figure 12: Real-Time PCR Primer Design.

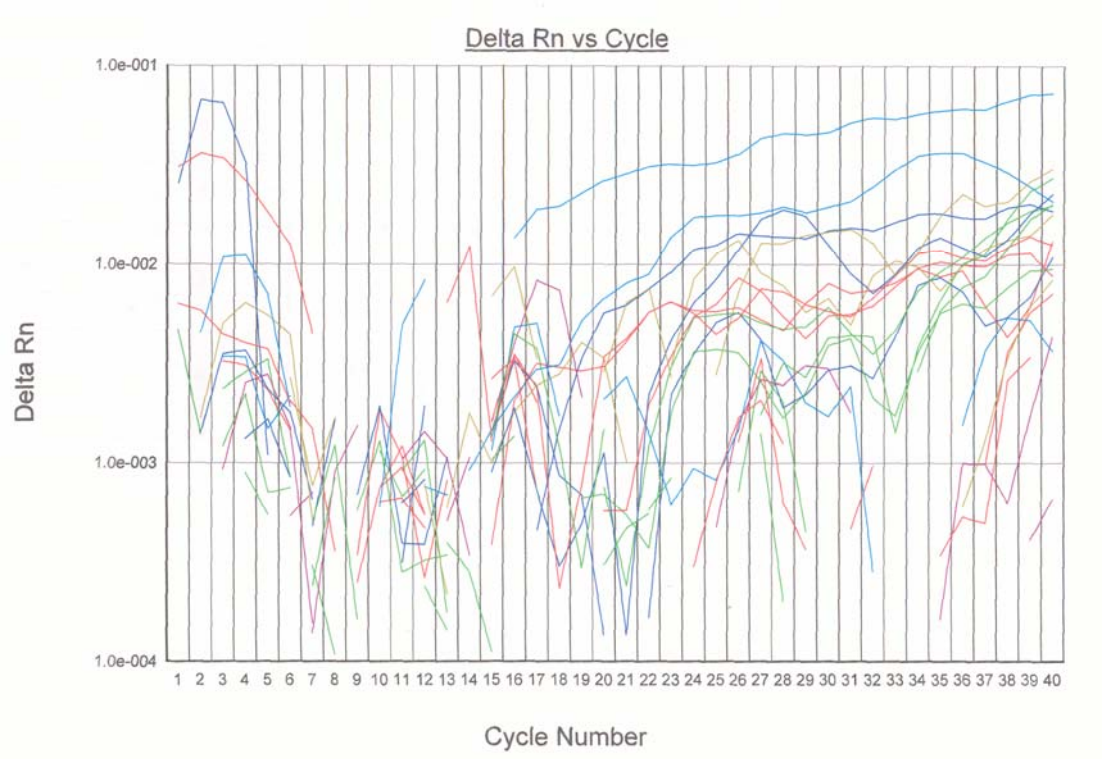
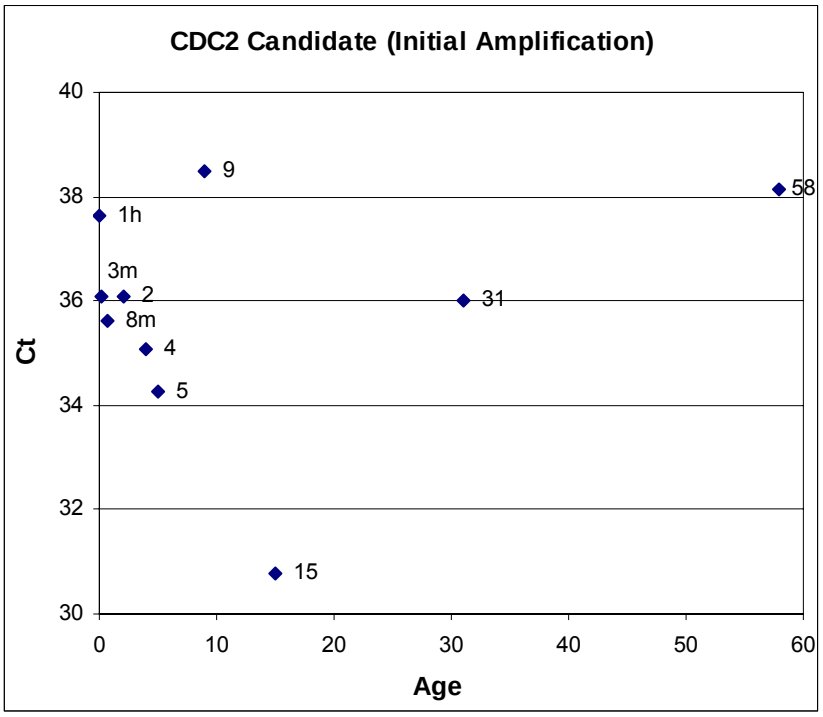
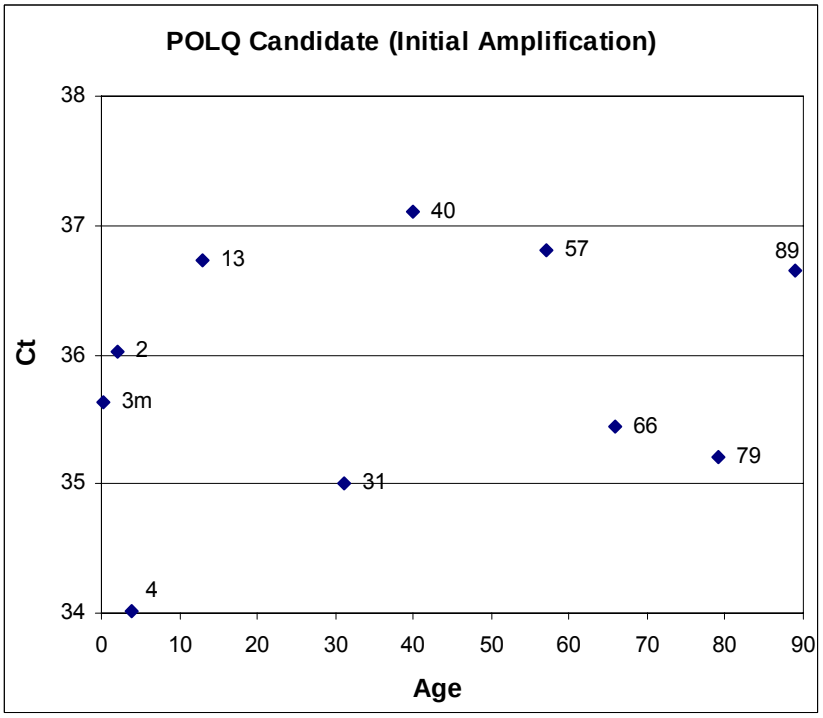
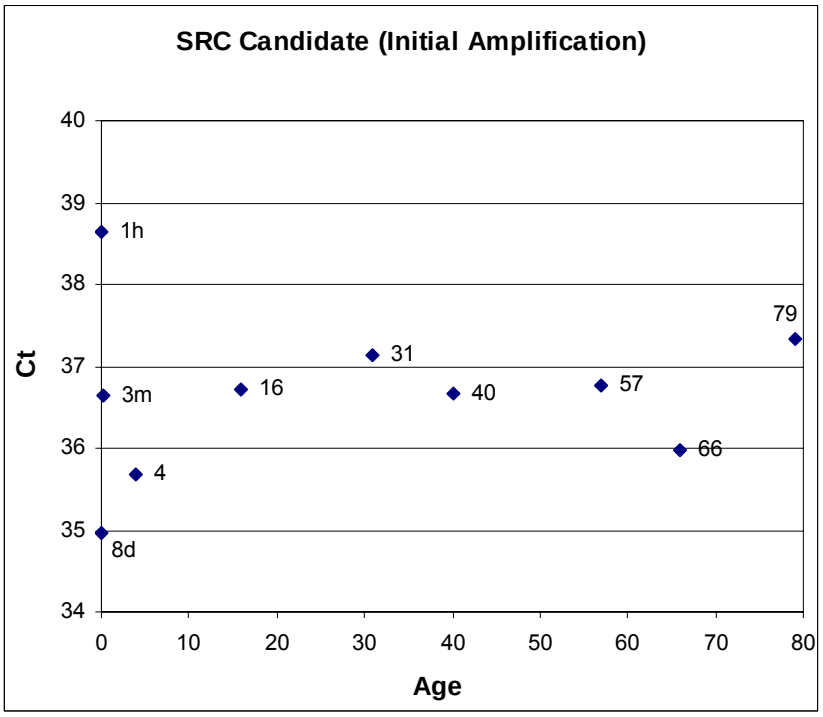
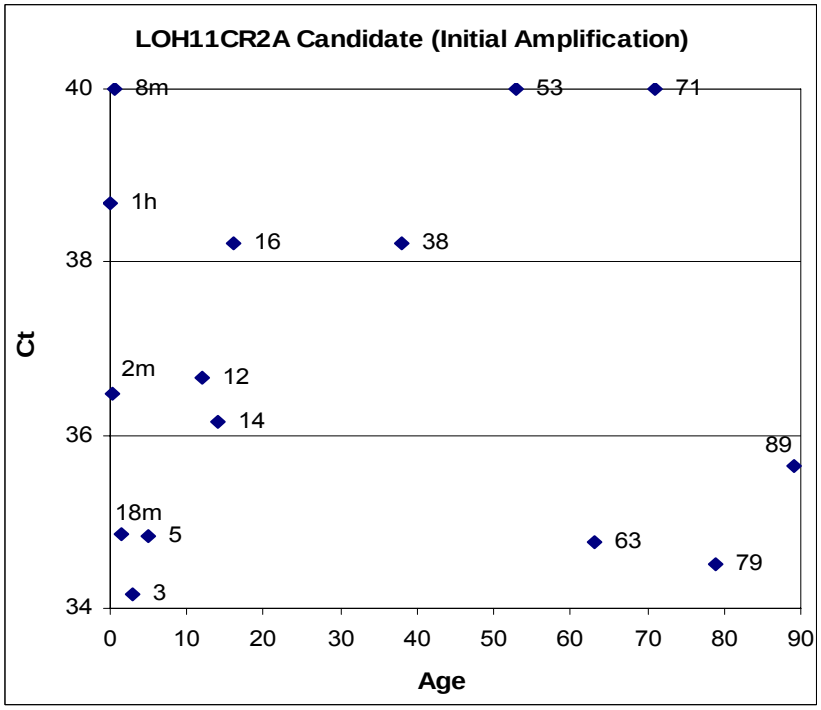


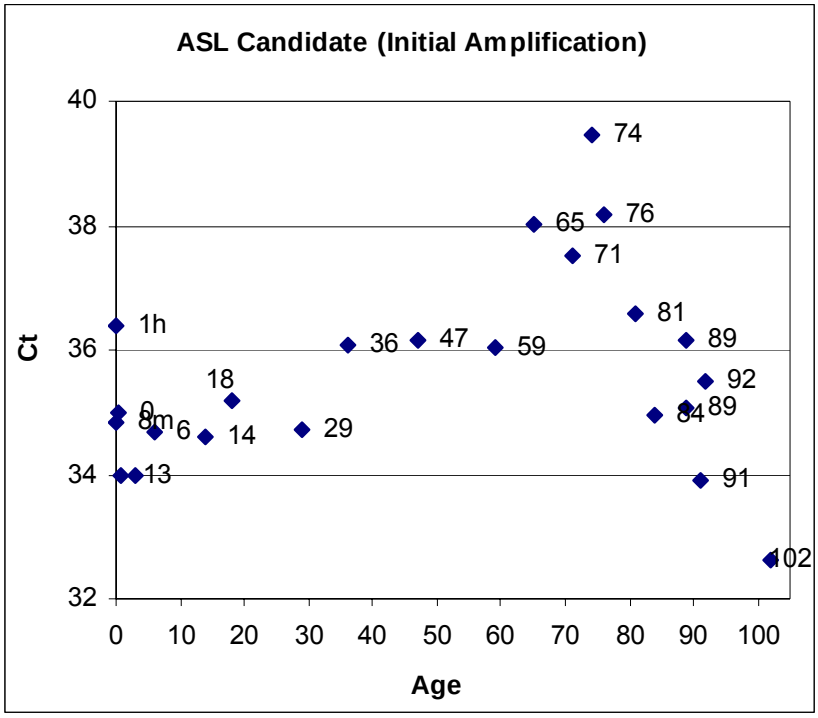
Figure 13: Amplification Results of the POLM Candidate Gene.

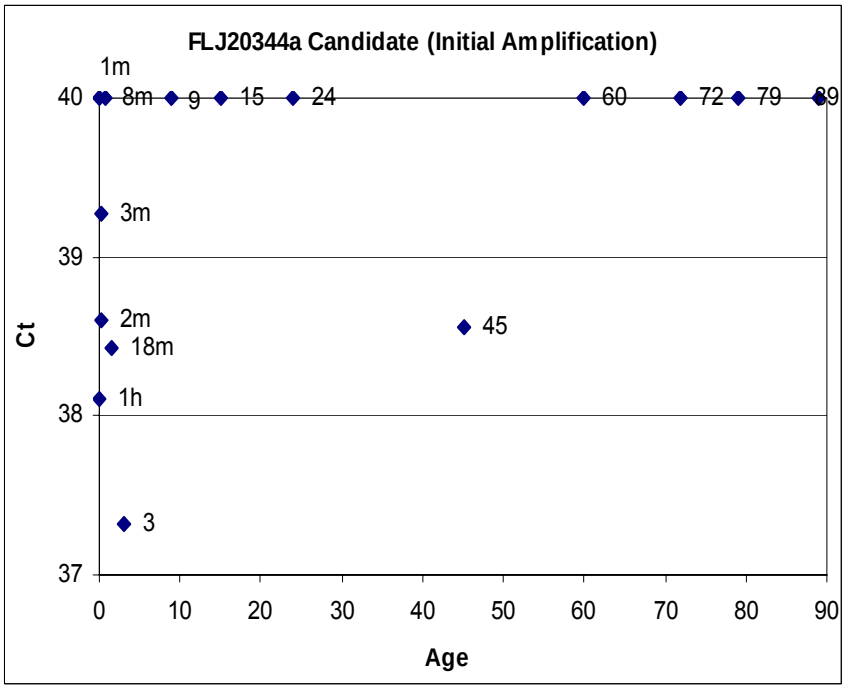

CDC2 Candidate (Initial Amplification)
Ct
Age
40
38
36
34
32
30
0
10
20
30
40
50
60
9
58
1h
3m
2
31
8m
4
5
15

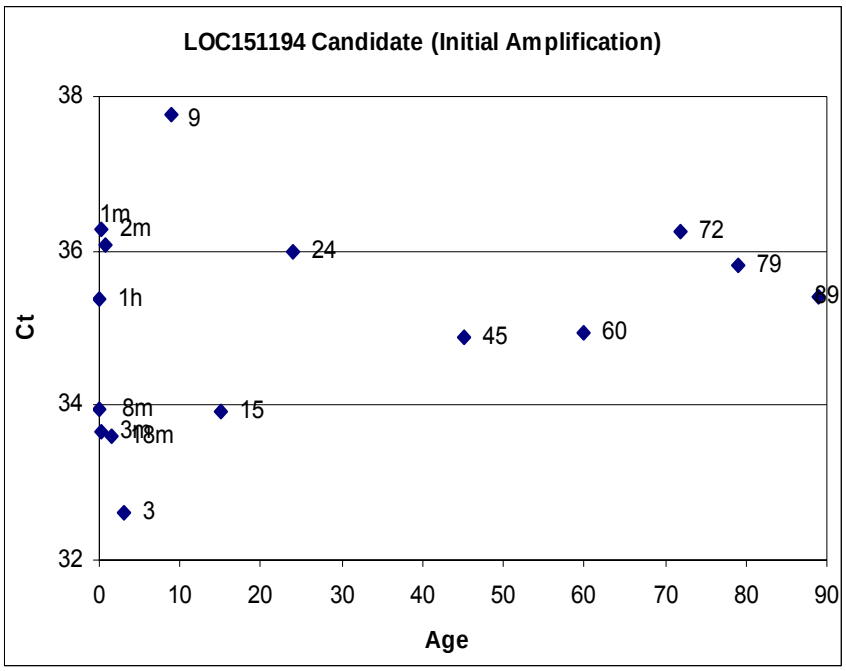

POLQ Candidate (Initial Amplification)
Ct
Age
38
37
36
35
34
0
10
20
30
40
50
60
70
80
90
40
57
89
13
2
3m
66
79
31
4

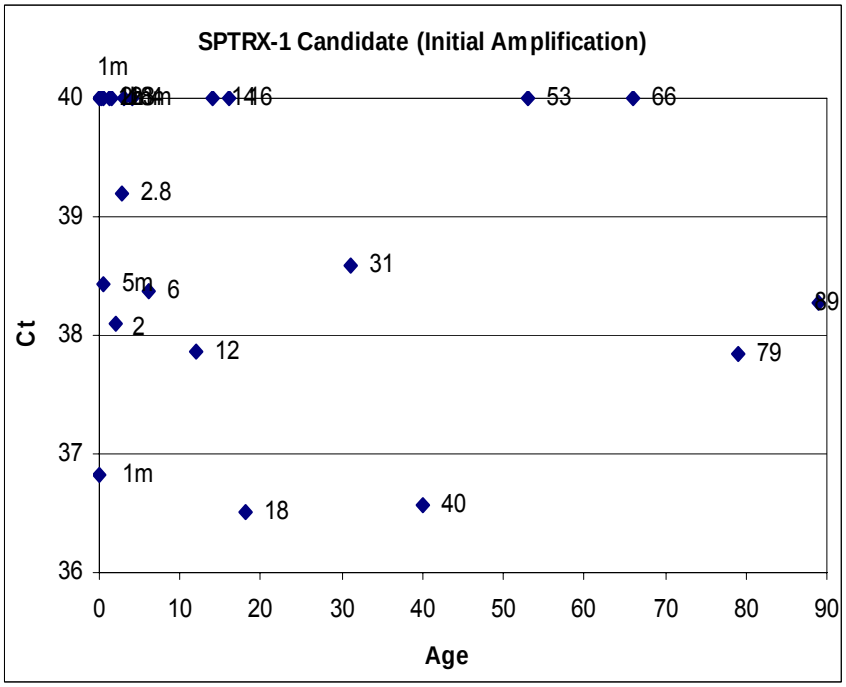

SRC Candidate (Initial Amplification)
Ct
Age
40
39
38
37
36
35
34
0
10
20
30
40
50
60
70
80
1h
3m
16
31
40
57
79
66
4
8d

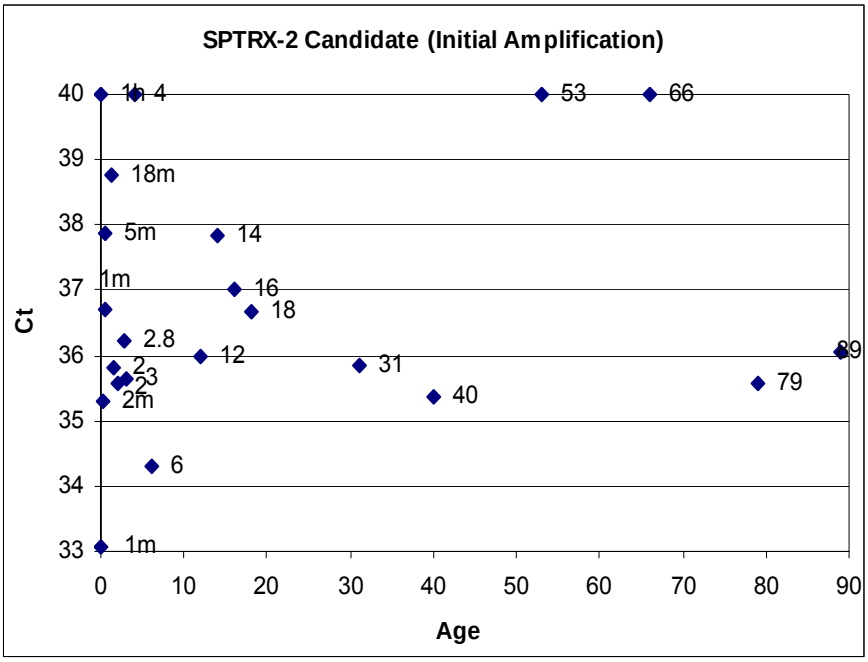

LOH11CR2A Candidate (Initial Amplification)
Ct
Age
40
38
36
34
0
10
20
30
40
50
60
70
80
90
8m
53
71
1h
16
38
2m
12
14
89
18m
5
63
79
3

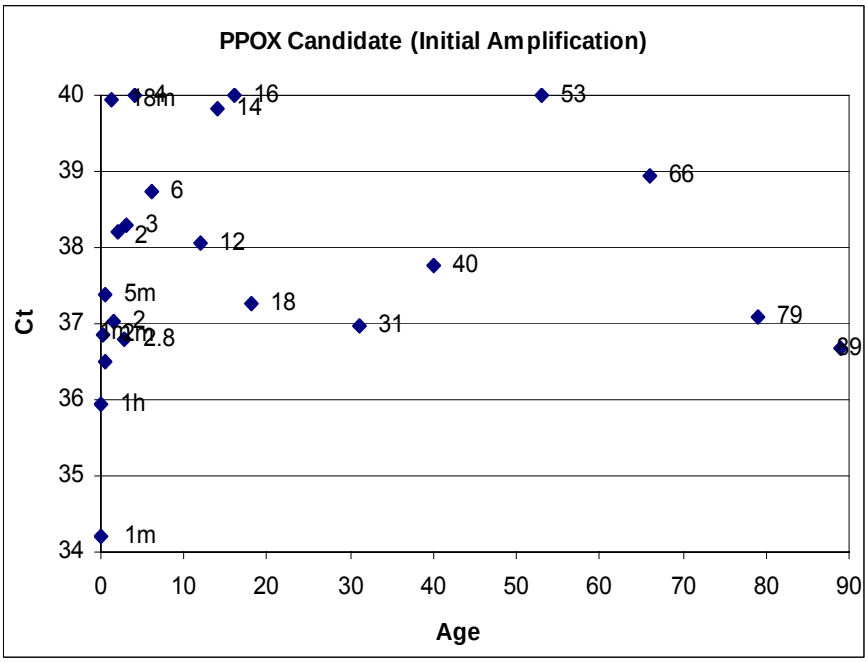

ASL Candidate (Initial Amplification)
Ct
Age
40
38
36
34
32
0
10
20
30
40
50
60
70
80
90
100
74
65
76
71
81
89
1h
36
47
59
18
92
89
0
8m
6
14
29
84
13
91
102

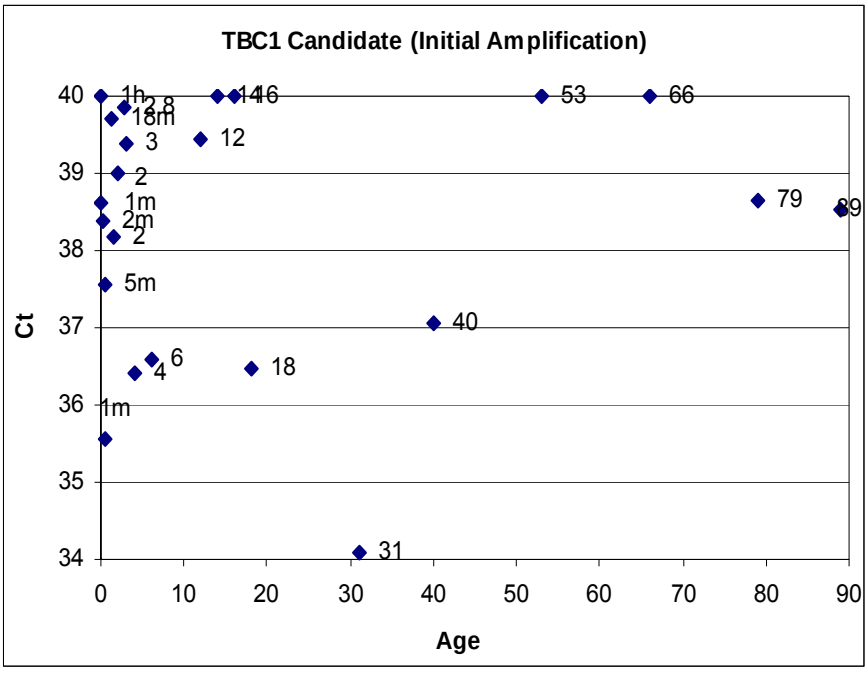

FLJ20344a Candidate (Initial Amplification)
Ct
Age
40
39
38
37
0
10
20
30
40
50
60
70
80
90
1m
8m
9
15
24
60
72
79
89
3m
2m
45
18m
1h
3

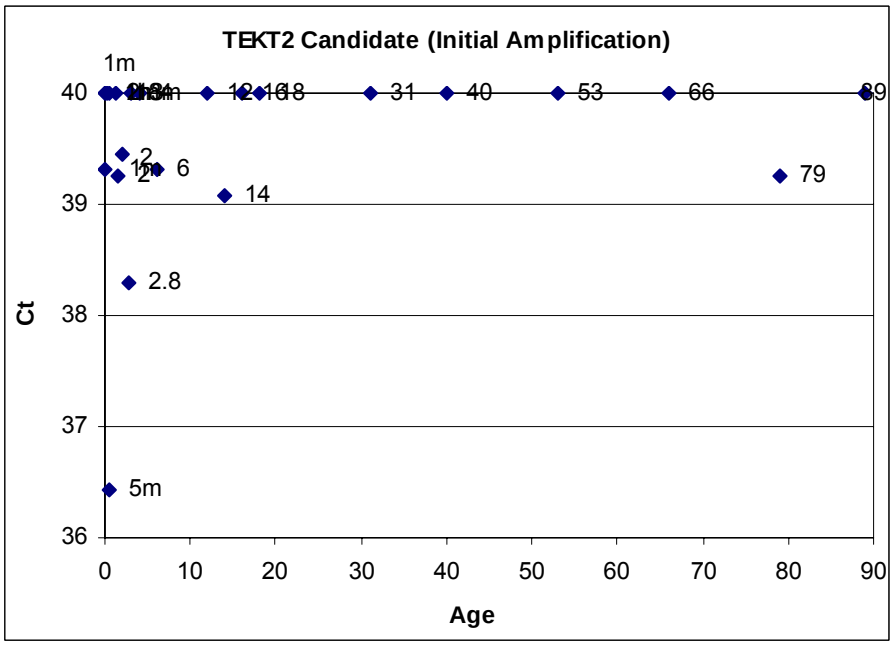

LOC151194 Candidate (Initial Amplification)
Ct
Age
38
36
34
32
0
10
20
30
40
50
60
70
80
90
9
1m
2m
24
72
79
89
1h
45
60
8m
15
3m
18m
3


SPTRX-1 Candidate (Initial Amplification)
Ct
Age
40
39
38
37
36
0
10
20
30
40
50
60
70
80
90
1m
53
66
2.8
31
5m
6
2
12
79
1m
18
40


SPTRX-2 Candidate (Initial Amplification)
Ct
Age
40
39
38
37
36
35
34
33
0
10
20
30
40
50
60
70
80
90
1h
4
53
66
18m
5m
14
1m
16
18
2.8
12
31
89
2
3
2m
40
79
6
1m


PPOX Candidate (Initial Amplification)
Ct
Age
40
39
38
37
36
35
34
0
10
20
30
40
50
60
70
80
90
4
16
53
18m
14
66
6
3
2
12
40
5m
18
79
2
31
2.8
89
1h
1m


TBC1 Candidate (Initial Amplification)
Ct
Age
40
39
38
37
36
35
34
0
10
20
30
40
50
60
70
80
90
1h
2.8
1&8m
3
12
2
1m
2m
2
5m
40
6
4
18
1m
31
53
66
79
89


TEKT2 Candidate (Initial Amplification)
Ct
Age
40
39
38
37
36
0
10
20
30
40
50
60
70
80
90
1m
2
6
14
2.8
5m
31
40
53
66
79
89

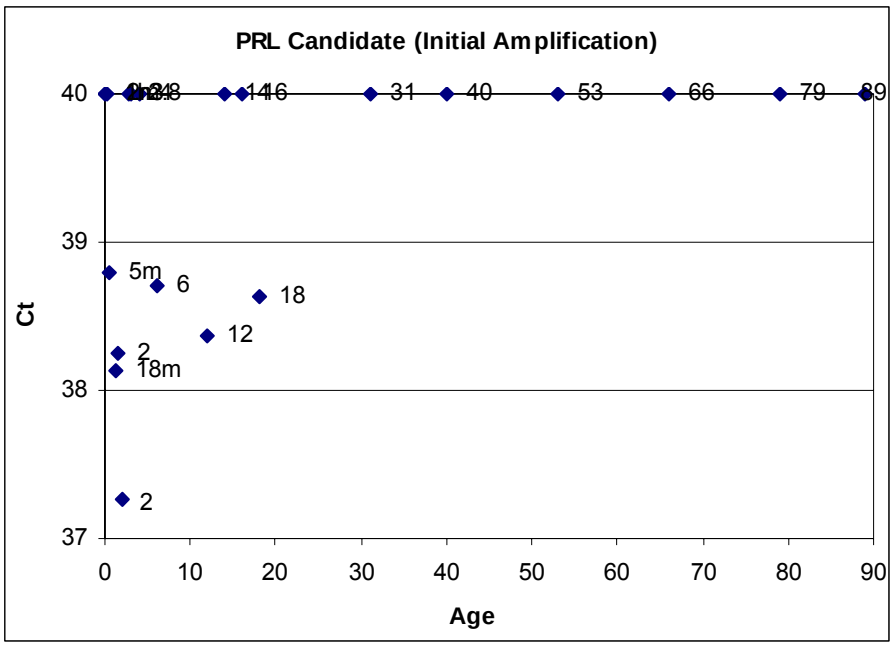
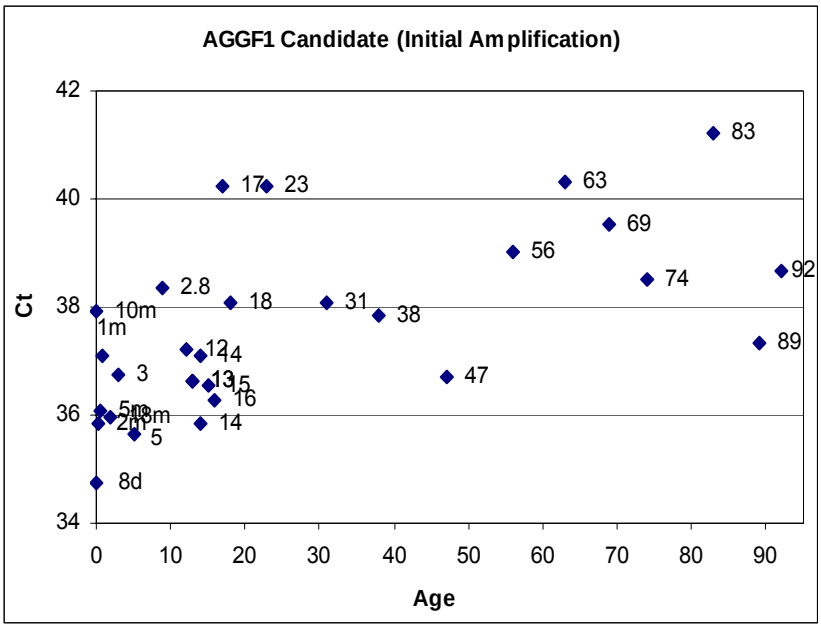
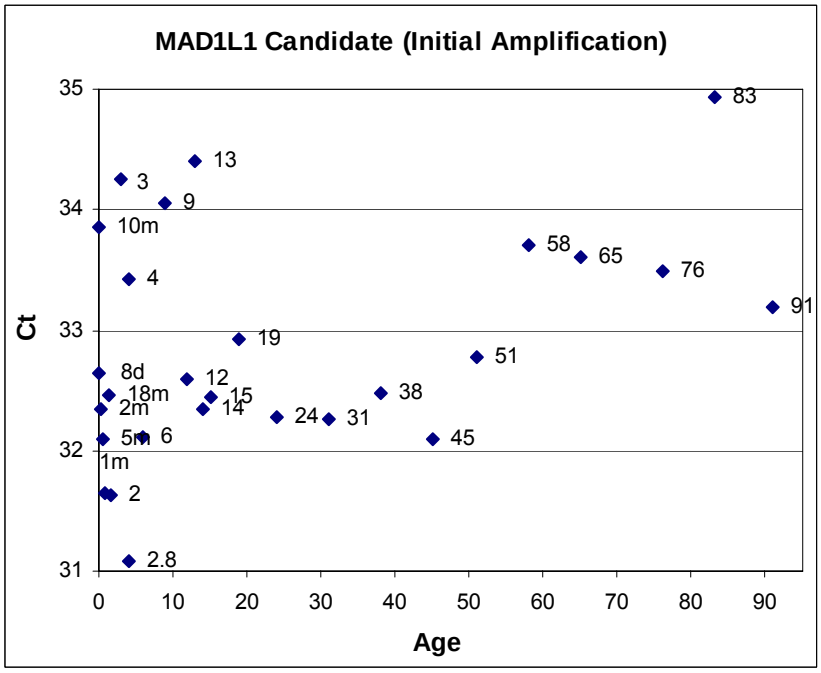
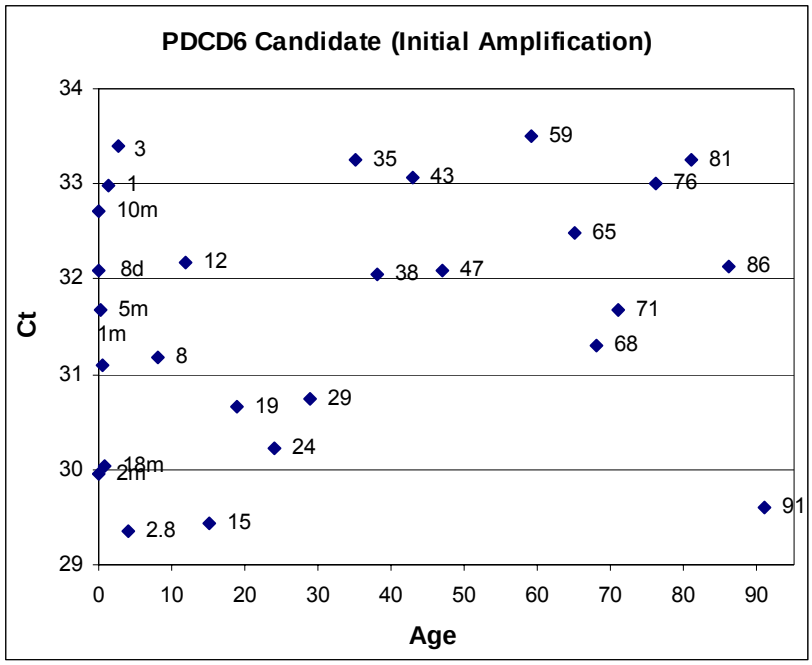


Figure 14: Real-Time PCR Candidate Gene Amplification Results.


AGGF1 Candidate (Initial Amplification)
Ct
Age
42
40
38
36
34
0
10
20
30
40
50
60
70
80
90
83
17
23
63
69
56
74
92
2.8
18
31
38
10m
1m
89
12
14
3
47
13
15
16
5m
18m
2m
14
5
8d


MAD1L1 Candidate (Initial Amplification)
Ct
Age
35
34
33
32
31
0
10
20
30
40
50
60
70
80
90
83
13
3
9
10m
58
65
76
4
91
19
51
8d
12
18m
15
38
2m
14
24
31
5m
6
45
1m
2
2.8



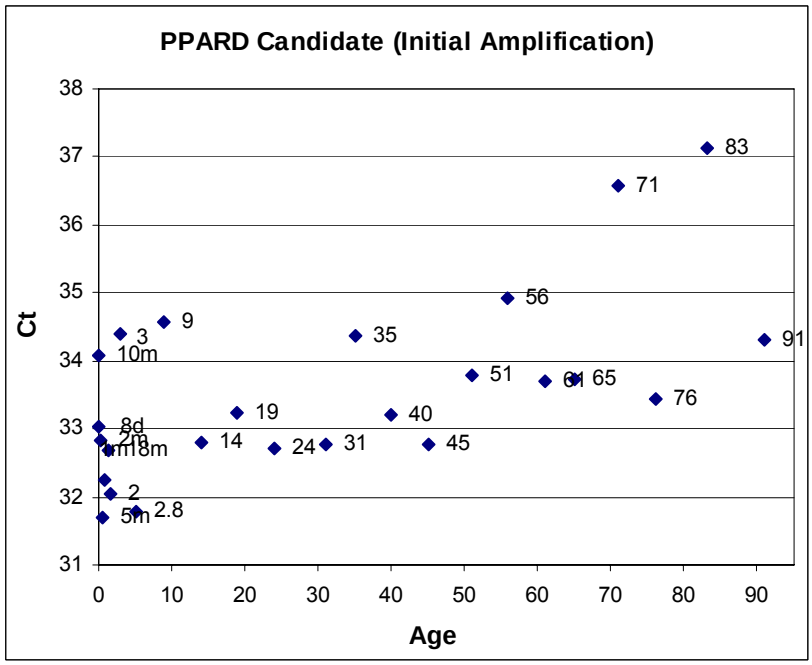
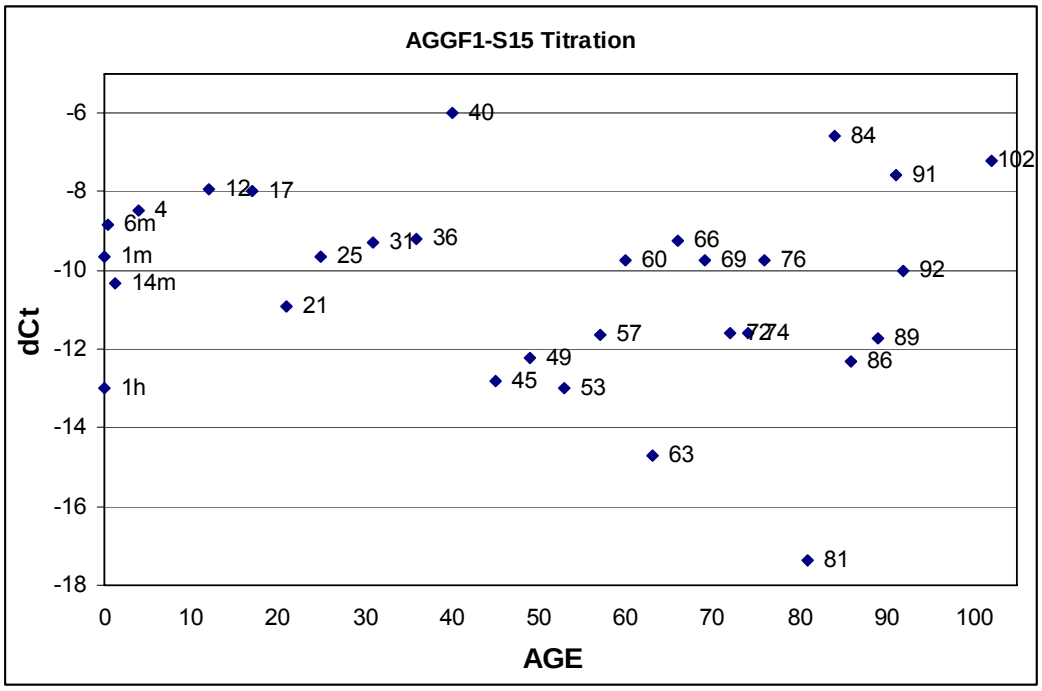
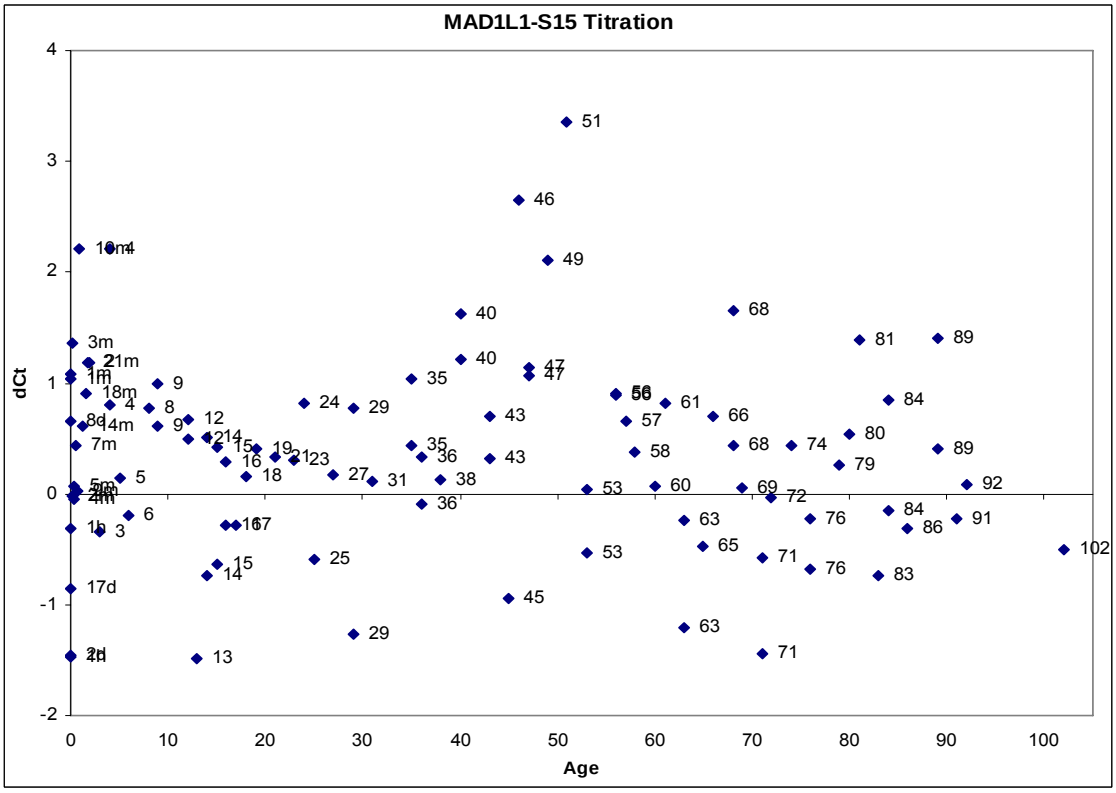
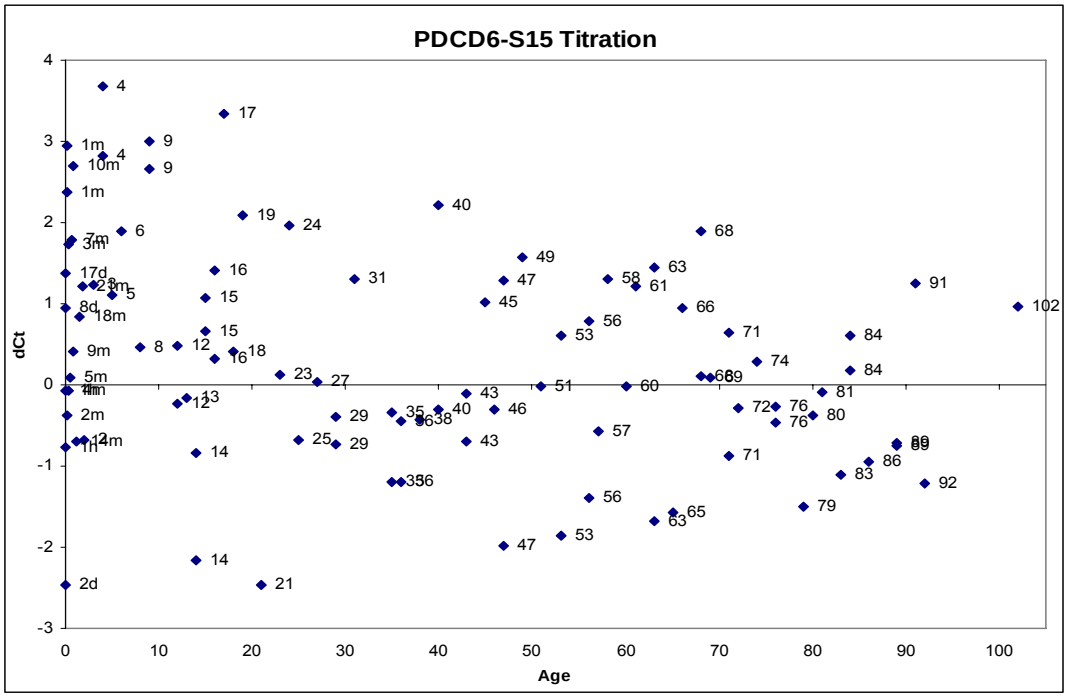


Figure 15: Real-Time PCR First-Round Candidate Results.


AGGF1-S15 Titration
dCt
AGE
-6
-8
-10
-12
-14
-16
-18
0
10
20
30
40
50
60
70
80
90
100
40
84
102
91
12
17
4
6m
1m
25
31
36
66
60
69
76
92
14m
21
57
89
49
86
45
53
1h
63
81


MAD1L1-S15 Titration
dCt
Age
4
3
2
1
0
-1
-2
0
10
20
30
40
50
60
70
80
90
100
51
46
49
40
68
81
89
3m
21m
9
35
24
29
43
57
61
66
84
18m
4
8
12
80
7m
58
68
74
89
79
5
18
27
31
38
53
60
69
72
92
6
3
63
76
84
86
91
102
65
71
76
83
17d
25
15
14
45
29
63
13
71



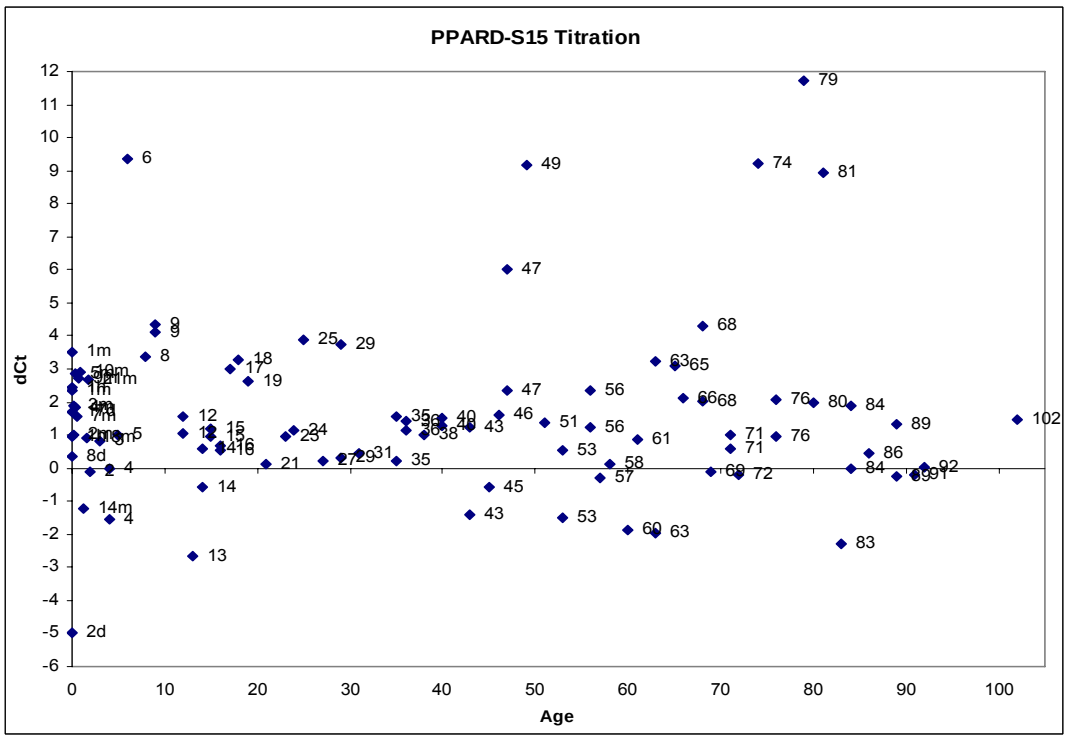


Figure 16: Real-Time PCR Duplex Delta Ct Results.

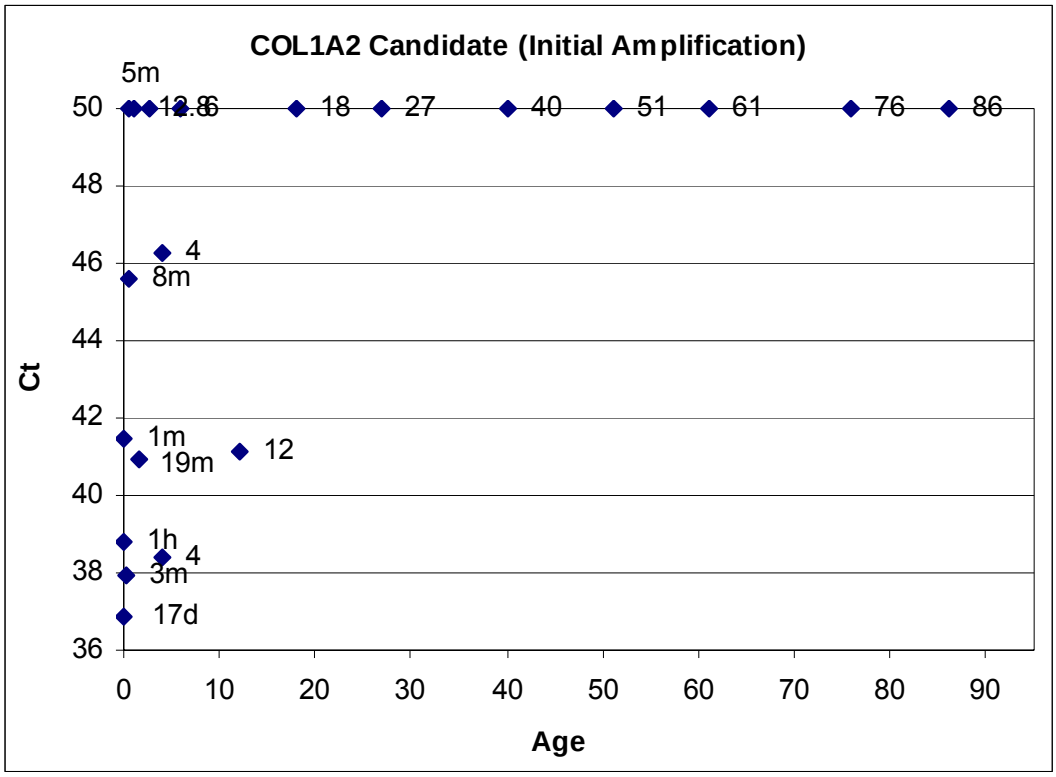


Figure 17: COL1A2 Real-Time PCR Singleplex Candidate Results.

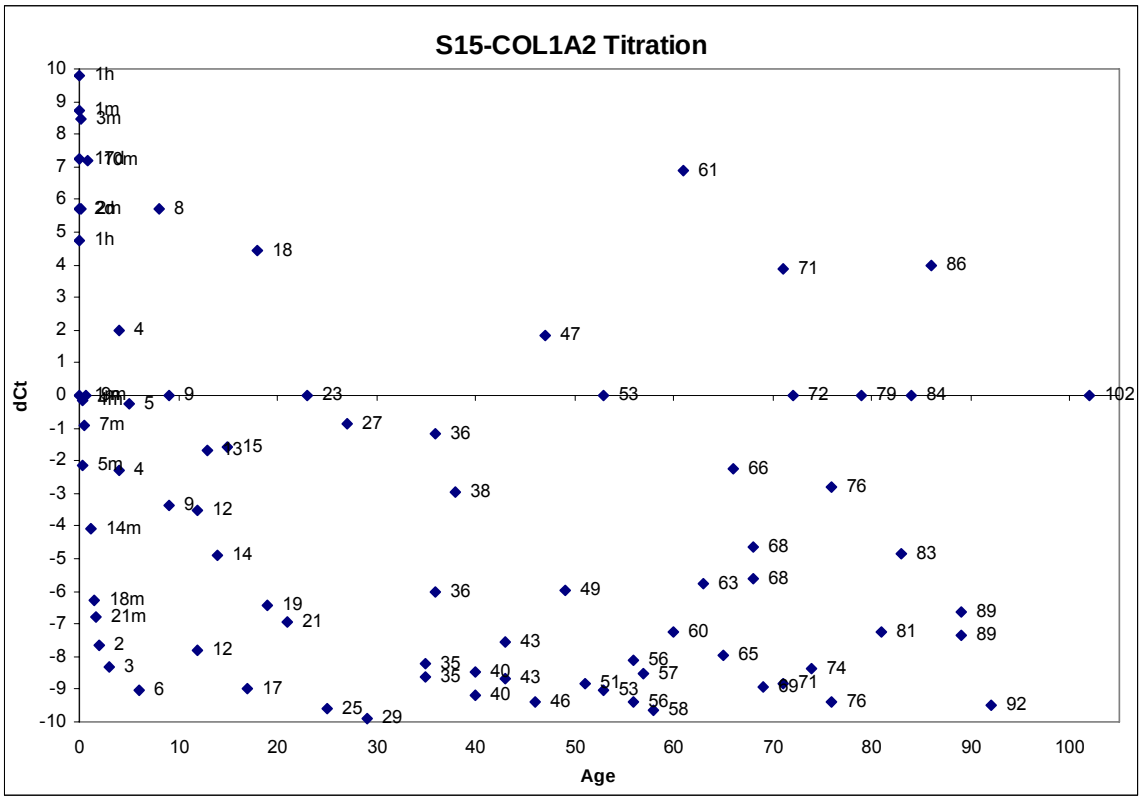
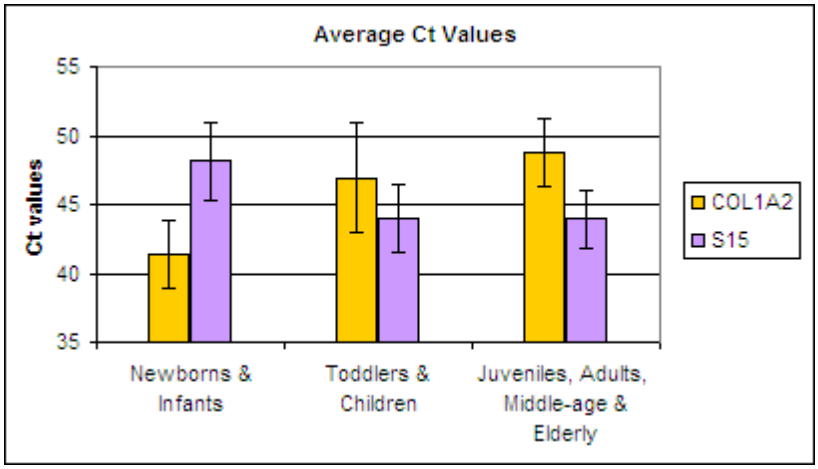


Figure 18: COL1A2 Real-Time PCR Duplex Delta Ct Results.

Average of all Samples per age group

Age Group	Average (GOI)	SD (GOI)	Average (S15)	SD (S15)	Average (dCt)	SD (dCt)	n=
Newborns & Infants	41.378	2.489	48.126	2.882	6.748	4.185	20
Toddlers & Children	46.928	4.008	43.948	2.497	-2.980	5.020	31
Juveniles, Adults, Middle-age & Elderly	48.751	2.491	43.921	2.052	-4.830	3.681	58



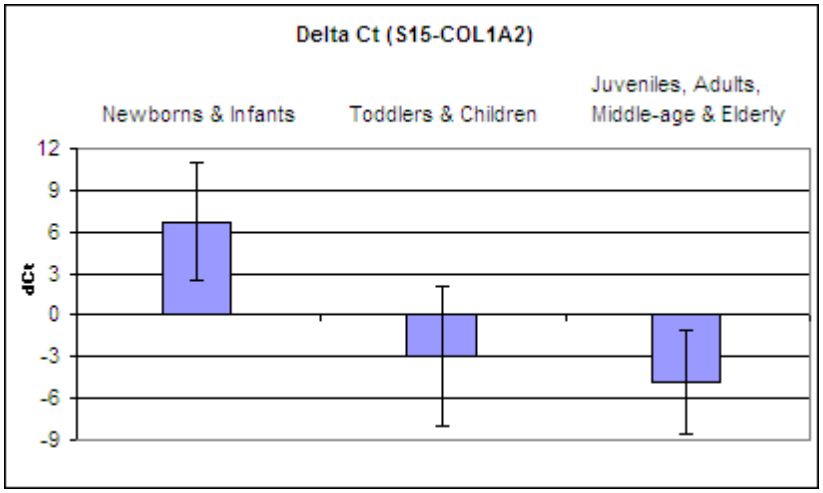


Figure 19: Newborn Candidate COL1A2 qPCR Duplex Biological Age Specificity.

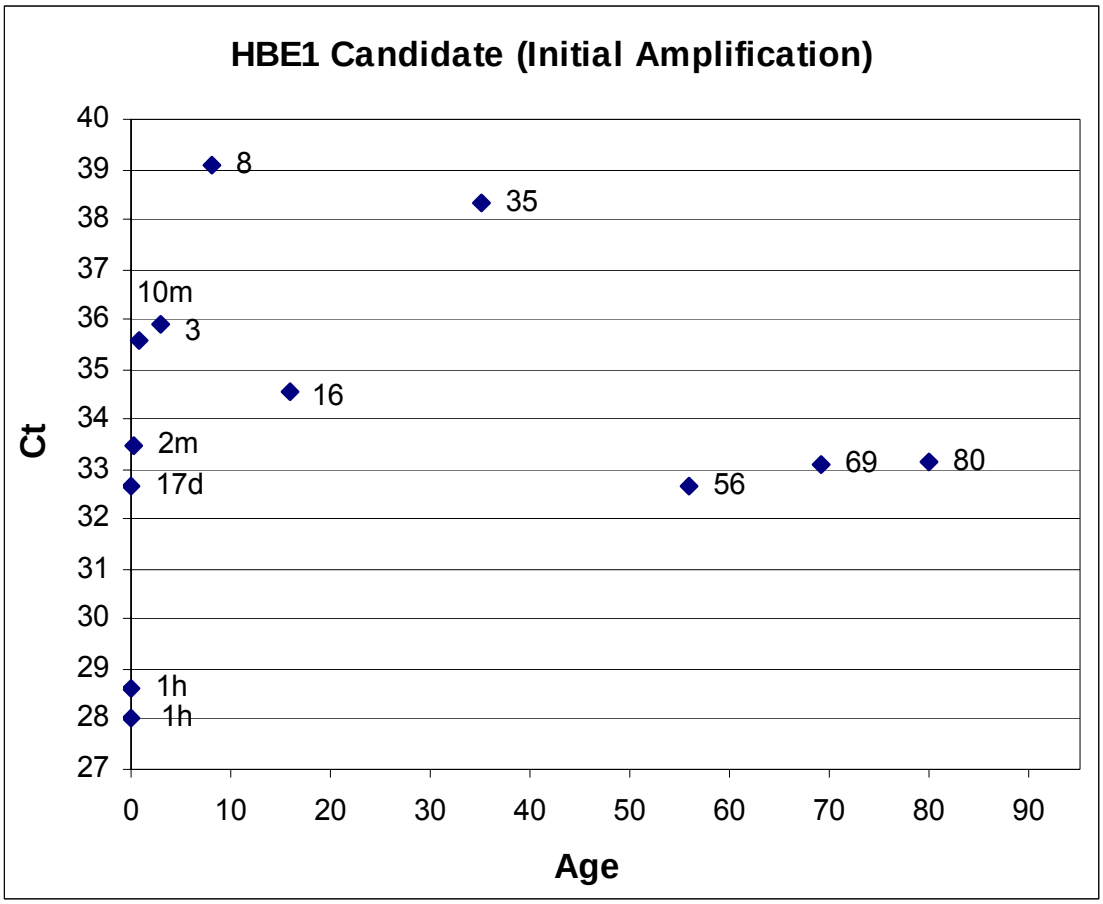


Figure 20: HBE1 Real-Time PCR Singleplex Candidate Results.

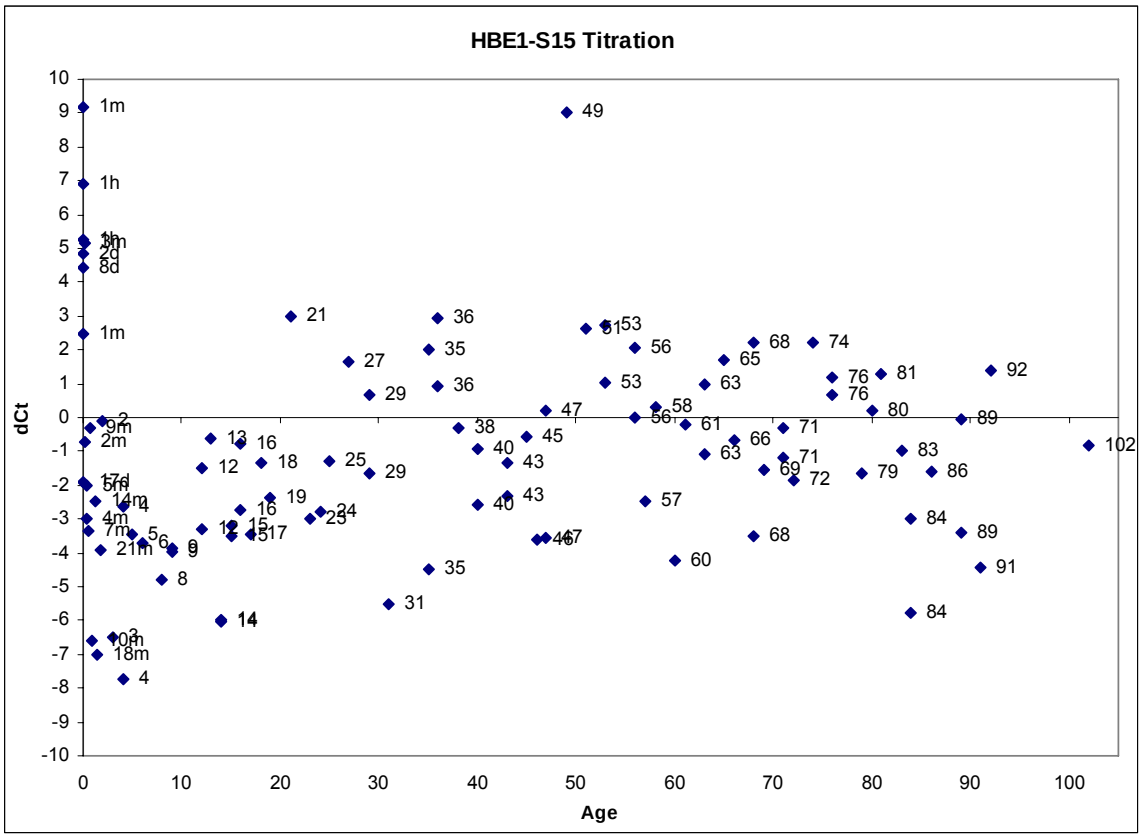
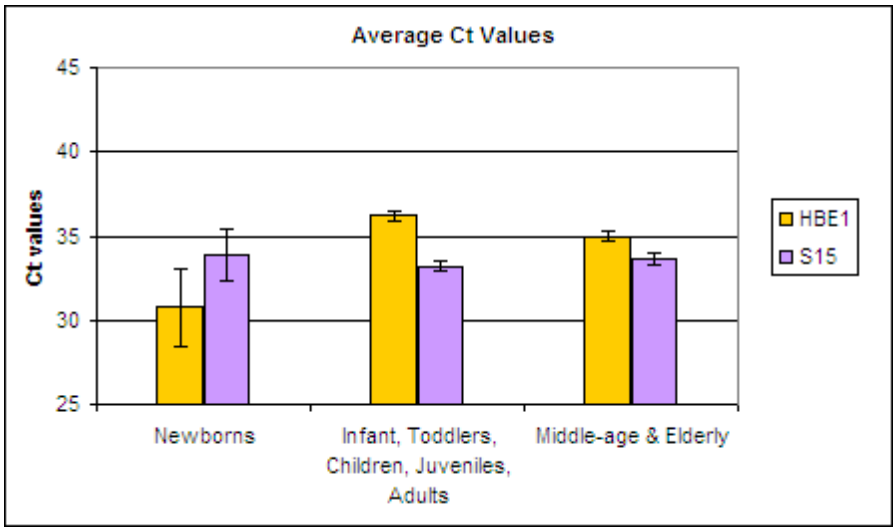


Figure 21: HBE1 Real-Time PCR Duplex Delta Ct Results.

Average of all Samples per age group

Age Group	Average (GOI)	SD (GOI)	Average (S15)	SD (S15)	Average (dCt)	SD (dCt)	n=
Newborns	30.750	2.270	33.838	1.531	3.088	2.523	17
Infant, Toddlers, Children, Juveniles, Adults	36.189	0.338	33.210	0.275	-2.978	0.302	81
Middle-age & Elderly	34.994	0.317	33.646	0.324	-1.348	0.330	41



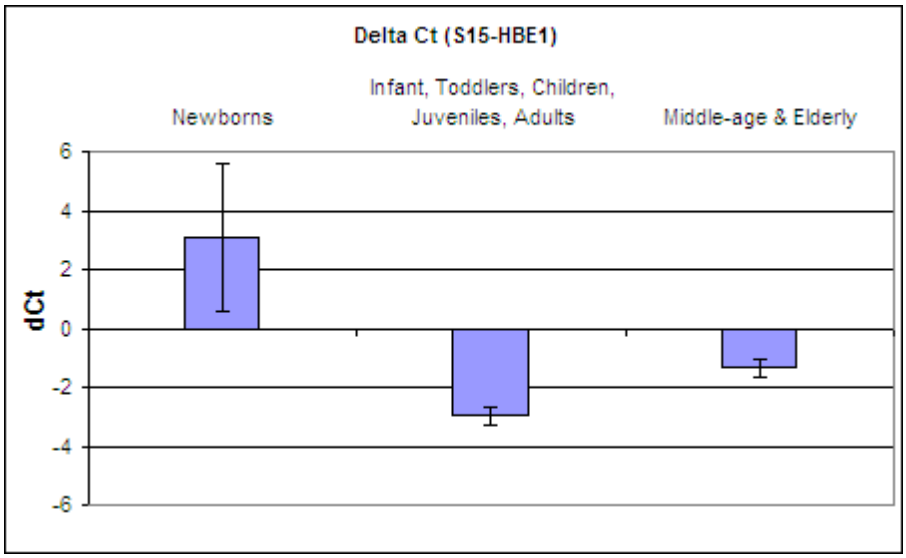


Figure 22: Newborn Candidate HBE1 qPCR Duplex Biological Age Specificity.

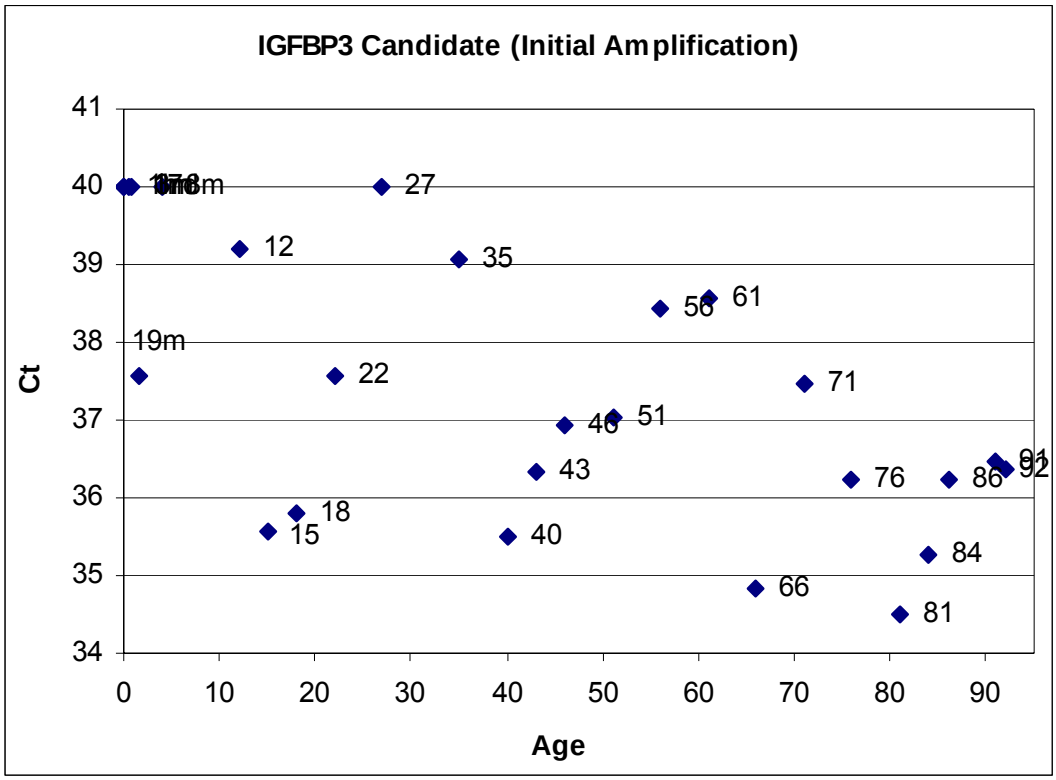


Figure 23: IGFBP3 Real-Time PCR Singleplex Candidate Results.

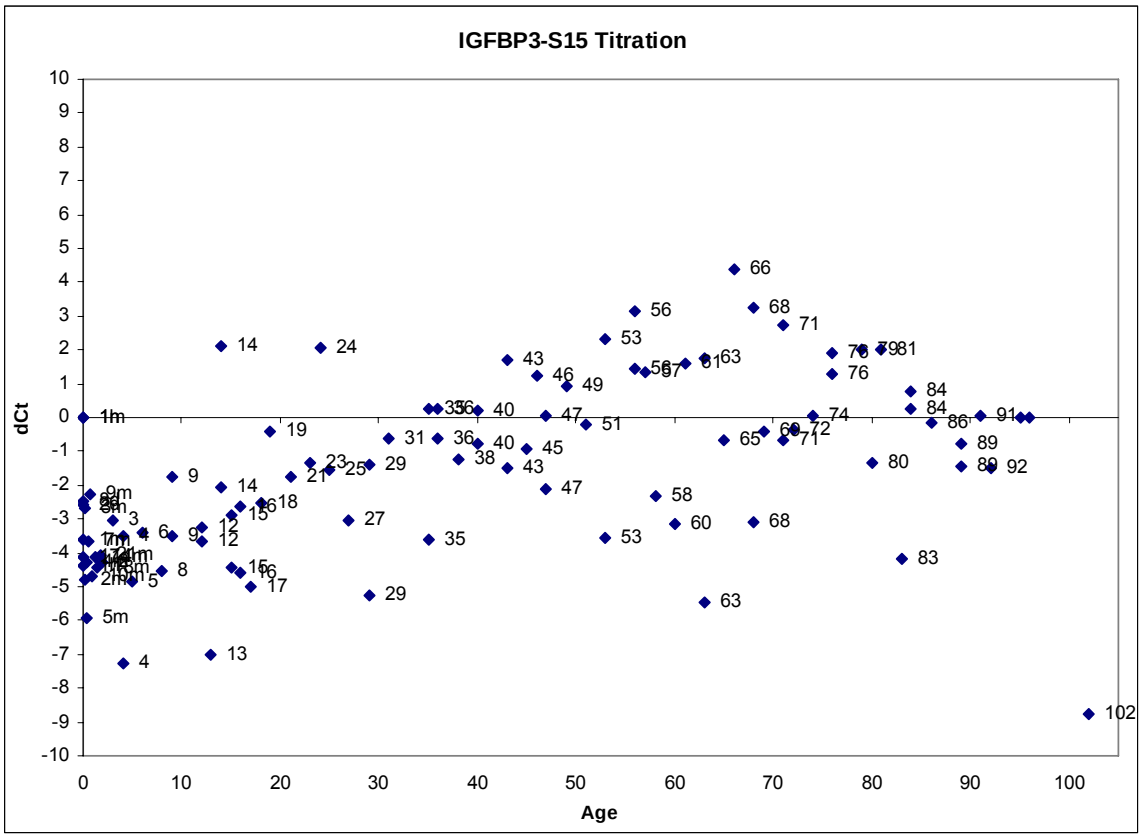
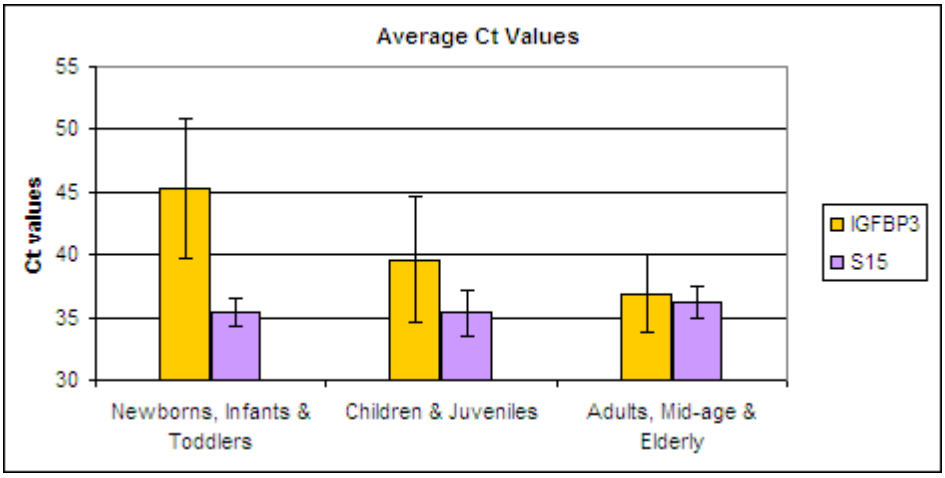


Figure 24: IGFBP3 Real-Time PCR Duplex Delta Ct Results.

Average of all Samples per age group

Age Group	Average (GOI)	SD (GOI)	Average (S15)	SD (S15)	Average (dCt)	SD (dCt)	n=
Newborns, Infants & Toddlers	45.298	5.576	35.384	1.131	-9.914	5.402	33
Children & Juveniles	39.604	5.033	35.396	1.818	-4.208	4.260	23
Adults, Mid-age & Elderly	36.914	3.143	36.189	1.331	-0.725	2.637	67



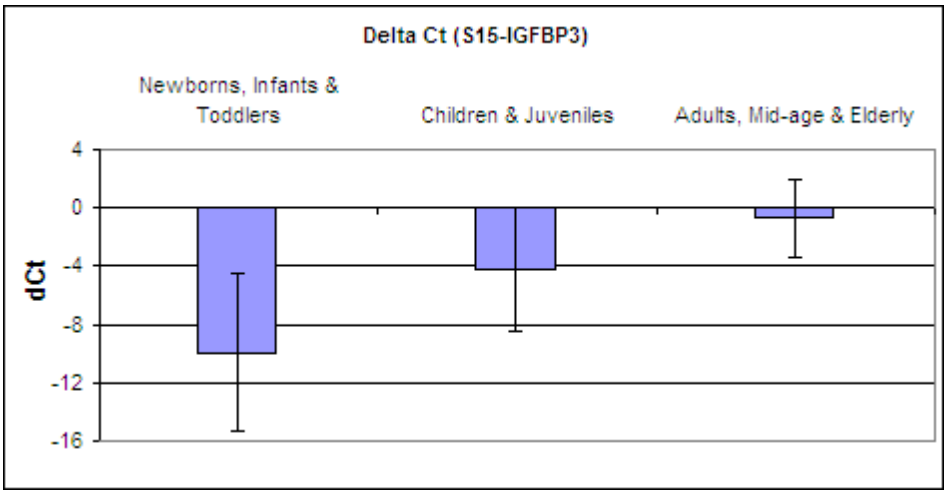


Figure 25: Post-pubertal Candidate IGFBP3 qPCR Duplex Biological Age Specificity.

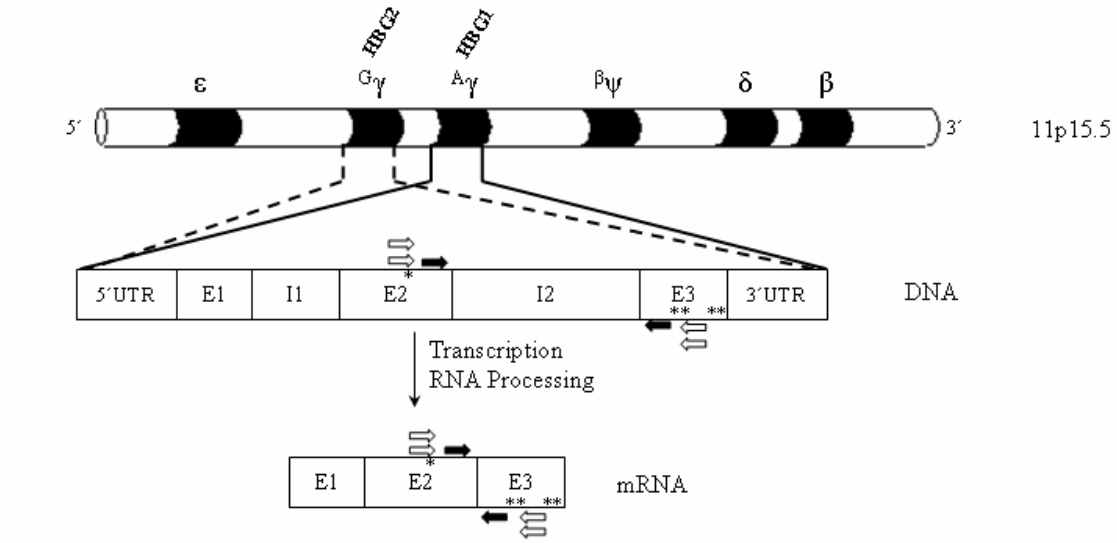


Figure 26: Structure of the Human Beta-Hemoglobin Locus.

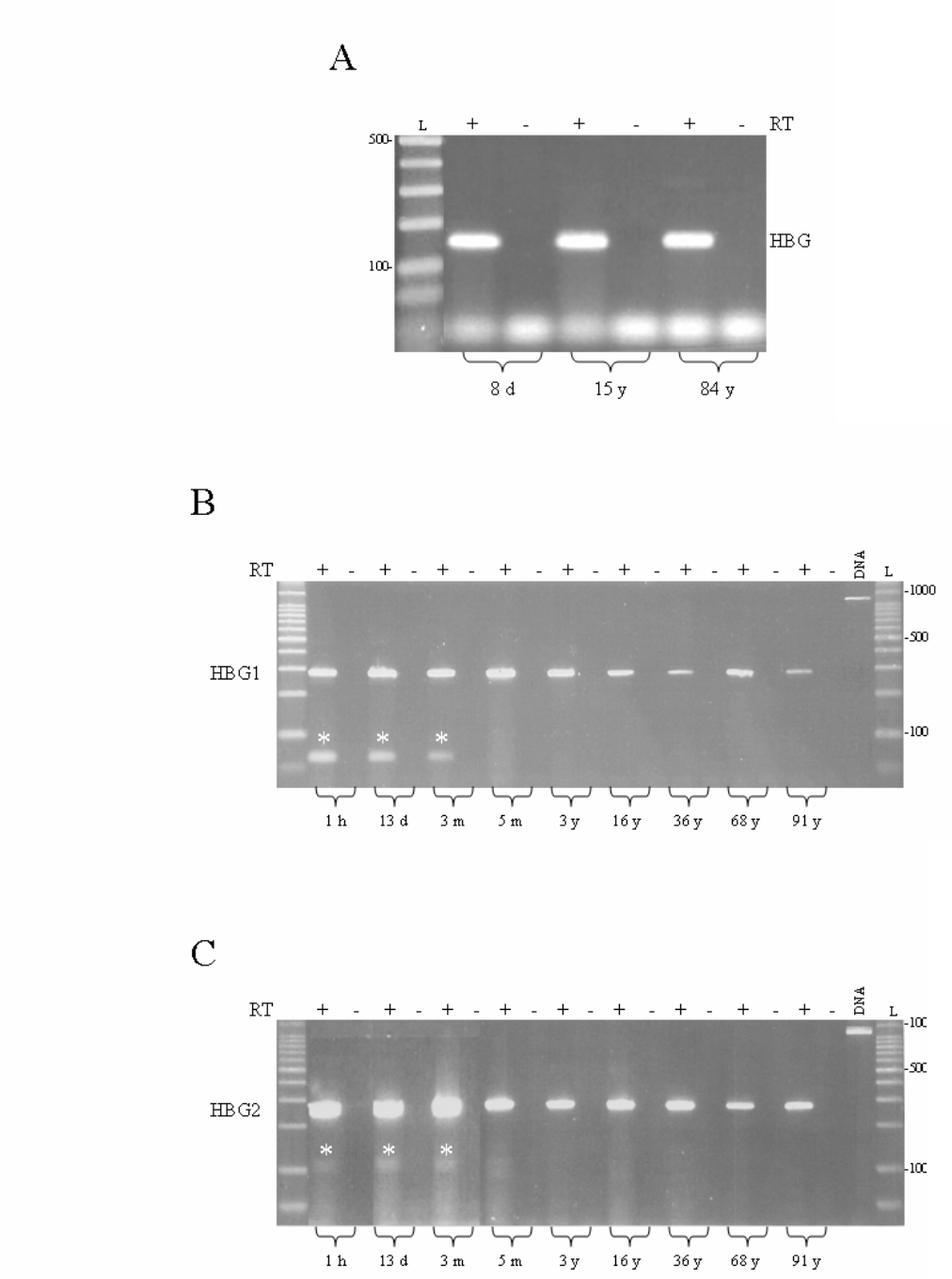


Figure 27: Identification of Gamma Hemoglobin Transcripts in Blood from Different Age Groups.

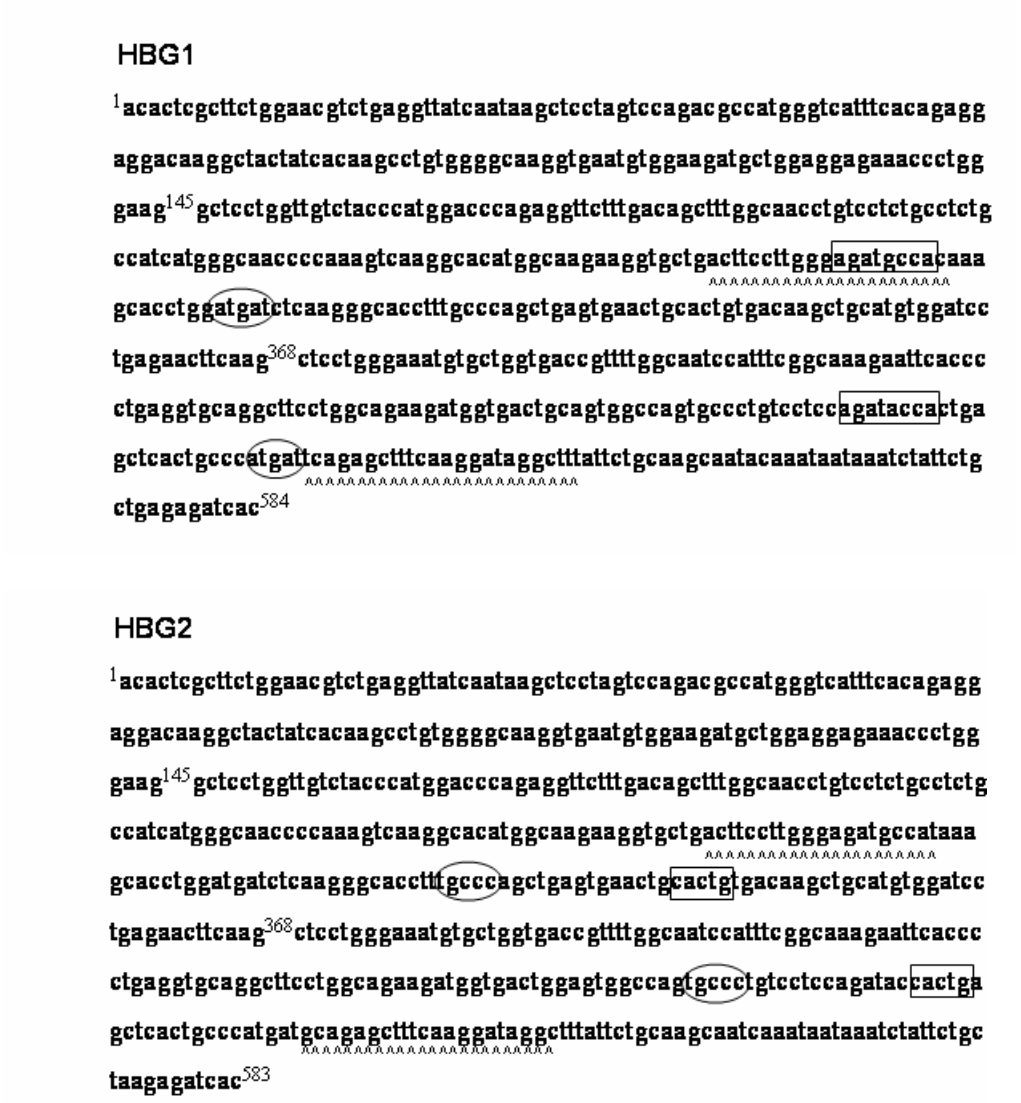


Figure 28: Standard mRNA Hemoglobin Sequences Identifying Newborn Specific Breakpoints.

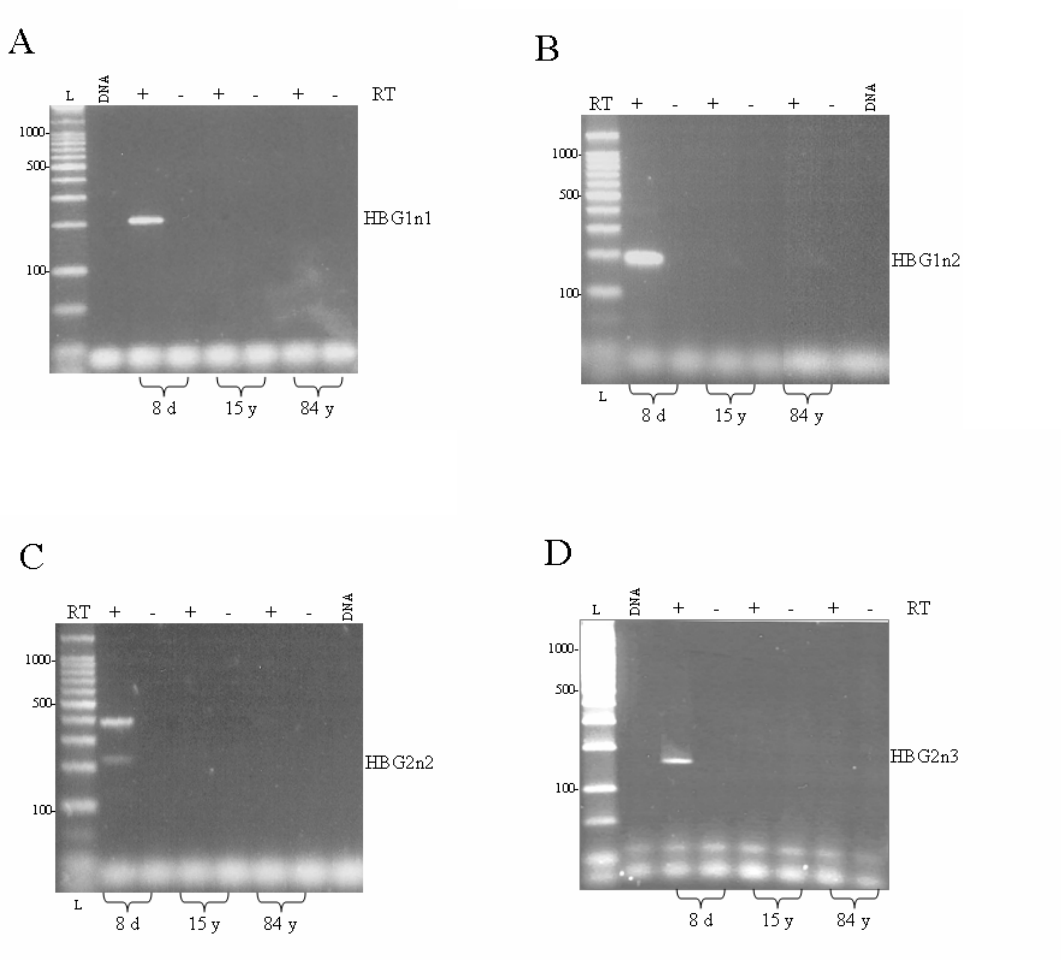


Figure 29: RT-PCR Amplification of Four Newborn-Specific Gene Transcripts.

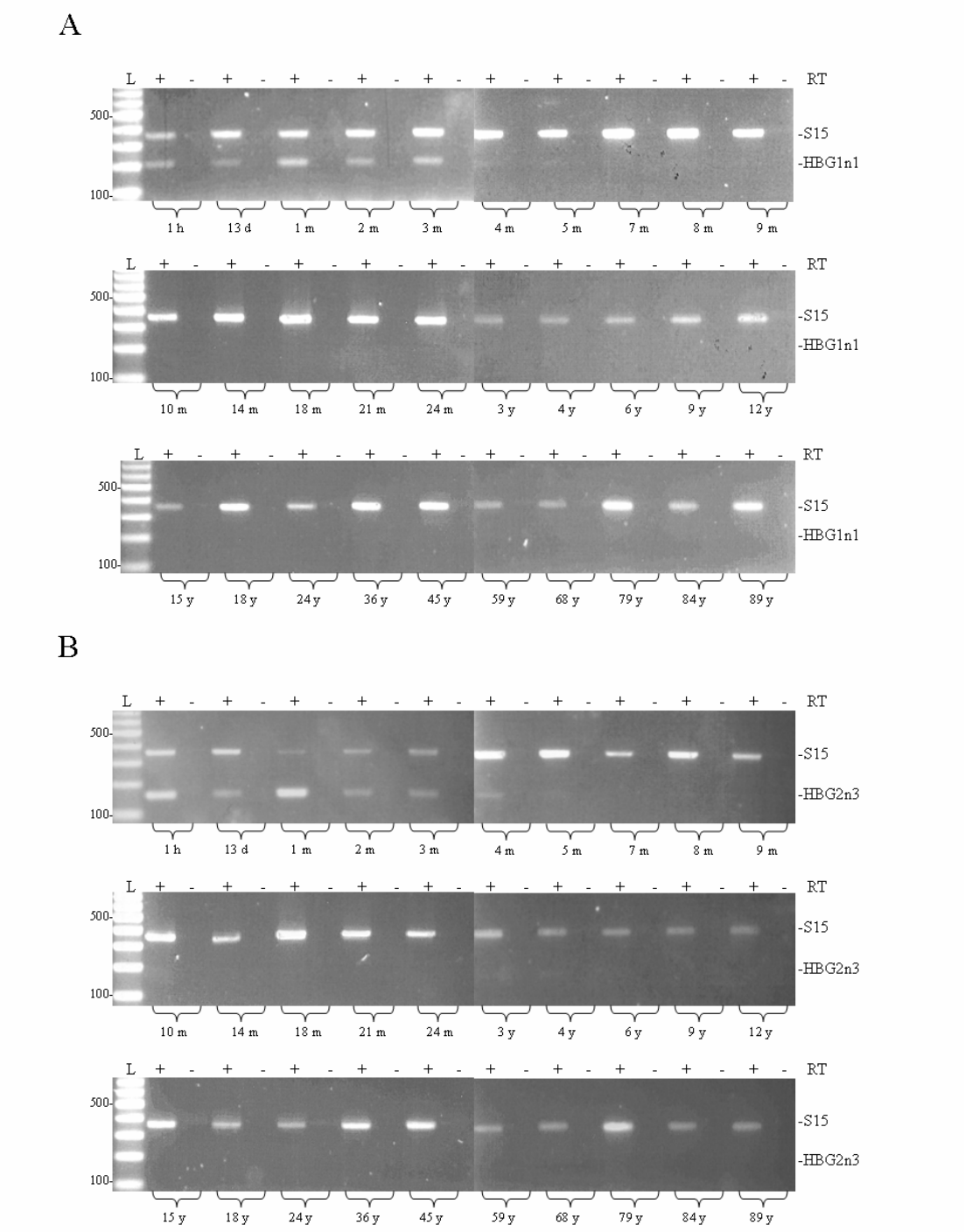


Figure 30: RT-PCR Based Age Specificity of the HBG1n1 and HBG2n3 Transcripts.

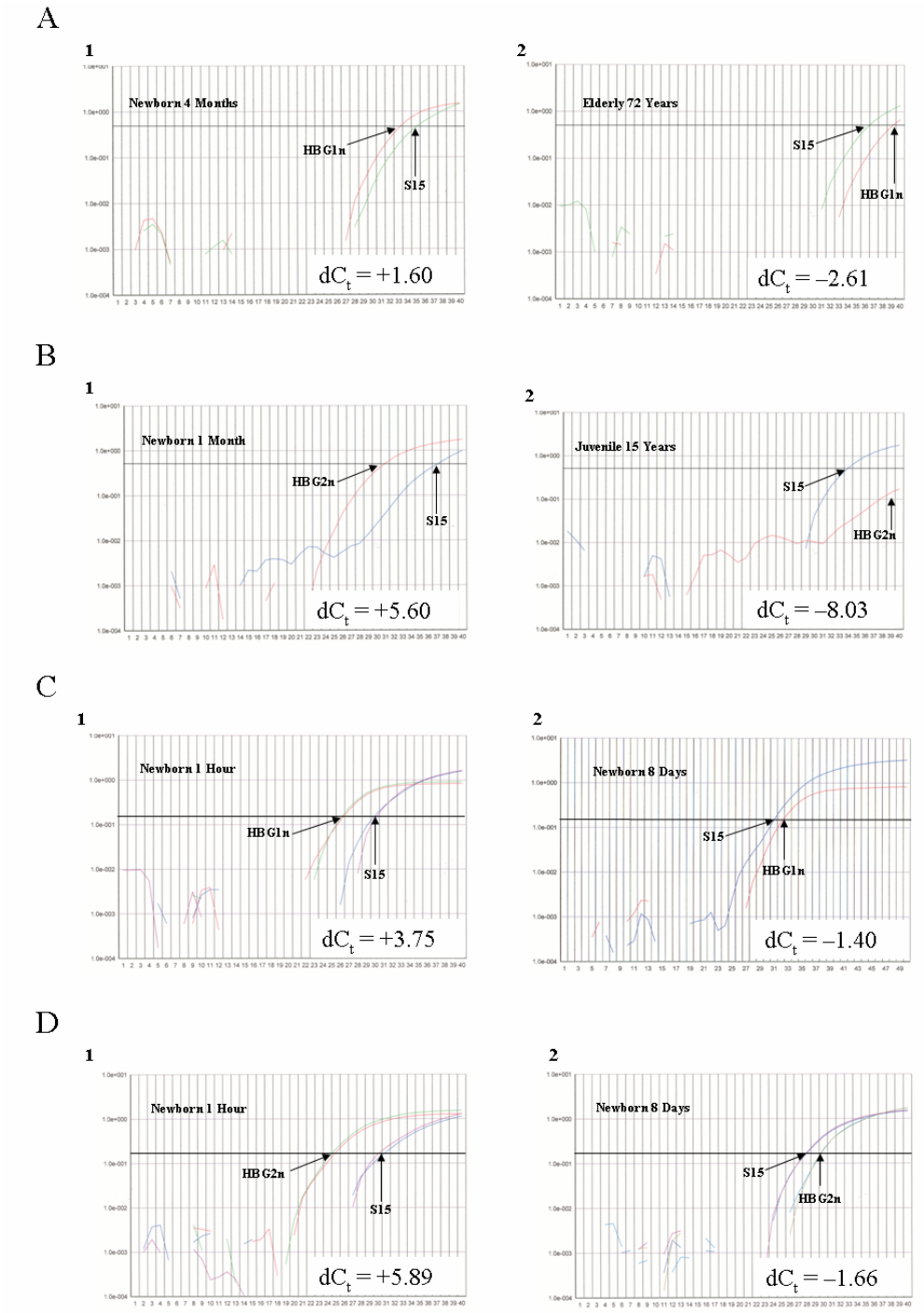


Figure 31: Quantitative Real-Time PCR Assays for the Identification of Newborns.

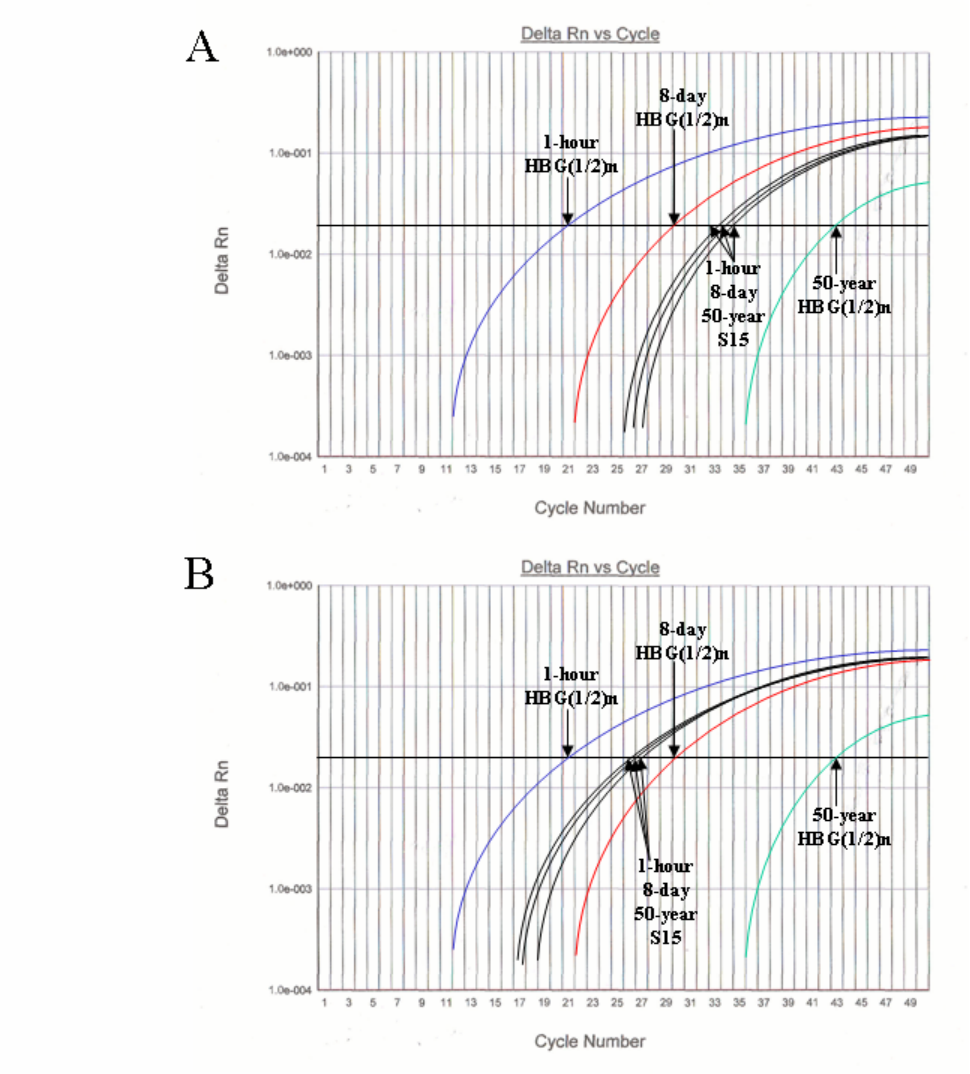
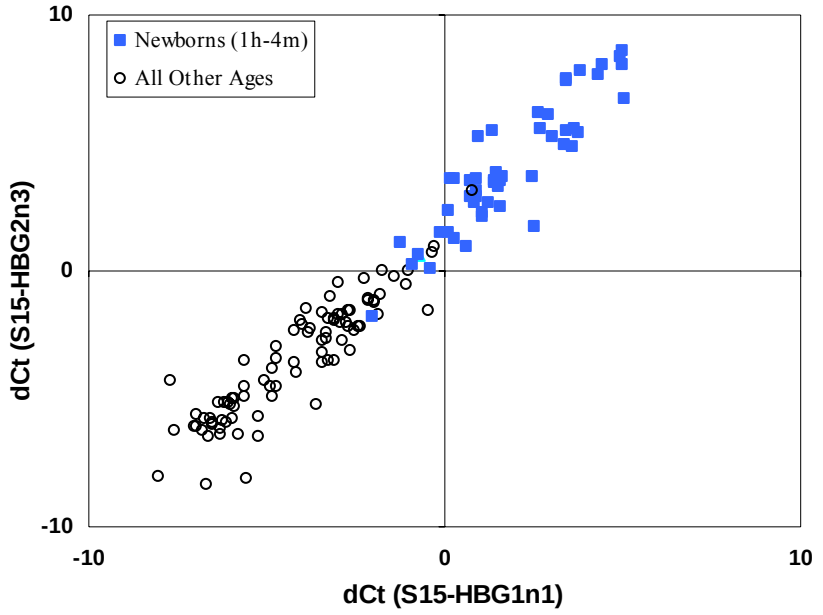


Figure 32: Delta Cycle Threshold Determination for Both Newborn Specific qPCR Assays.

A



B

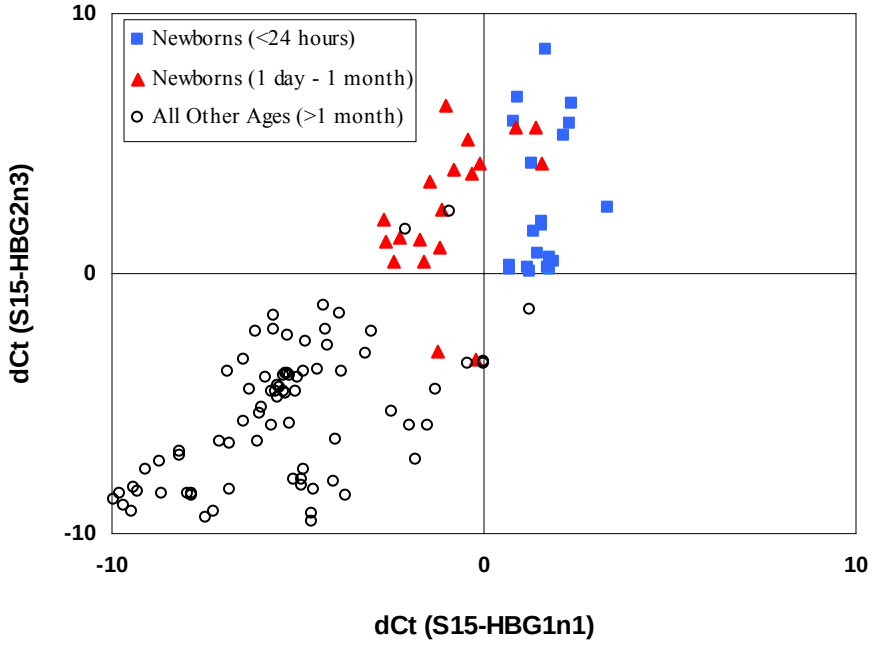
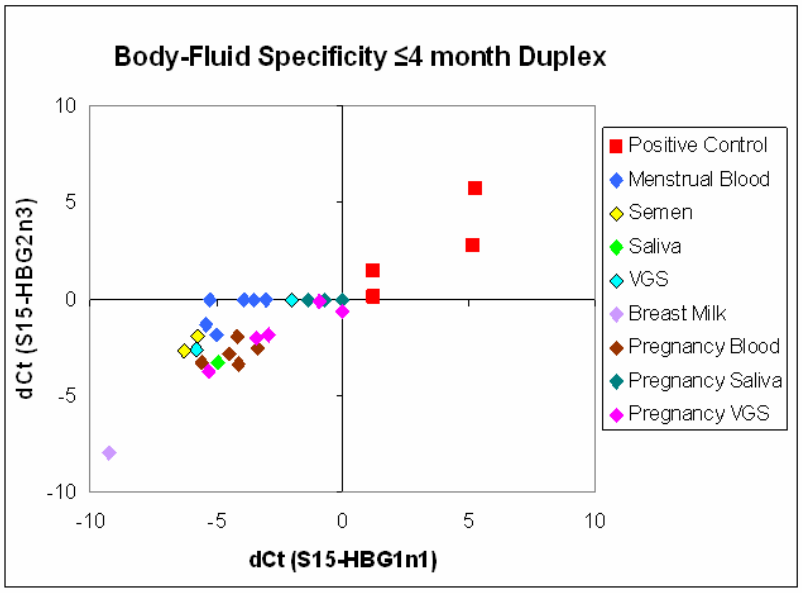


Figure 33: Biological Age Specificity of the HBG1n1 and HBG2n3 qRT-PCR Assays.

A



B

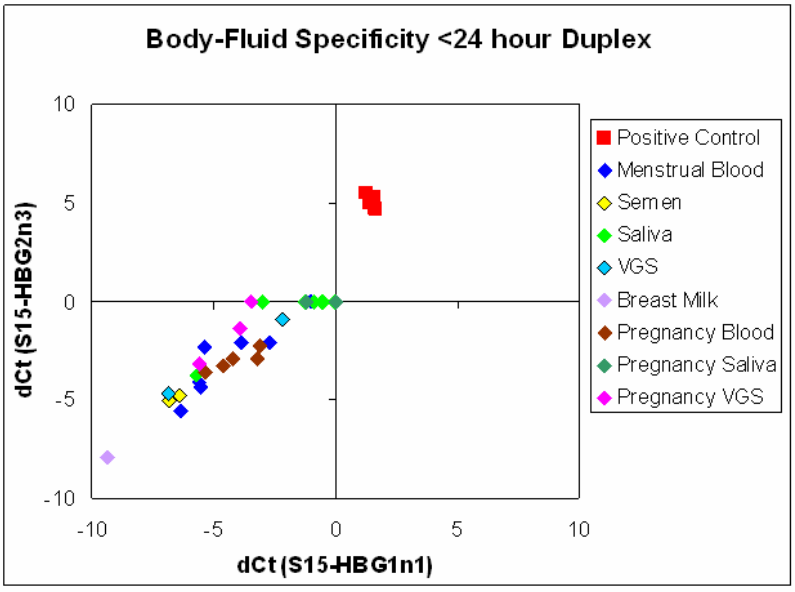
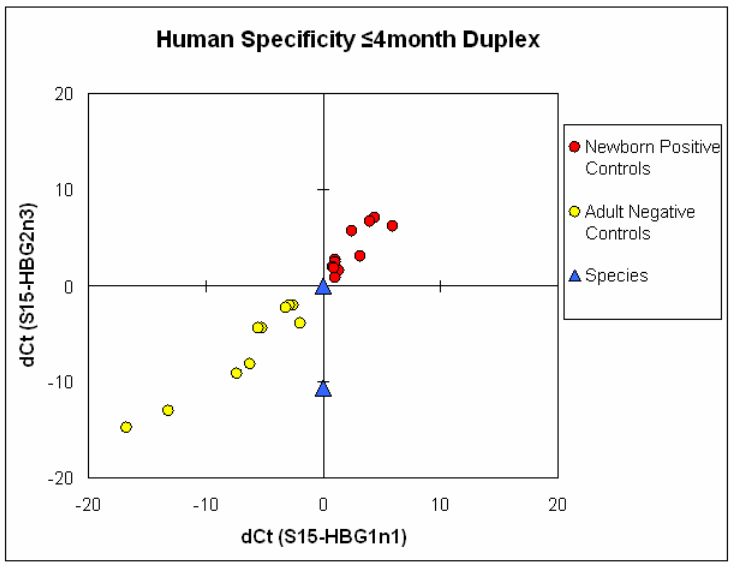


Figure 34: Body-Fluid Specificity for the Newborn Duplex Assays.

A



B

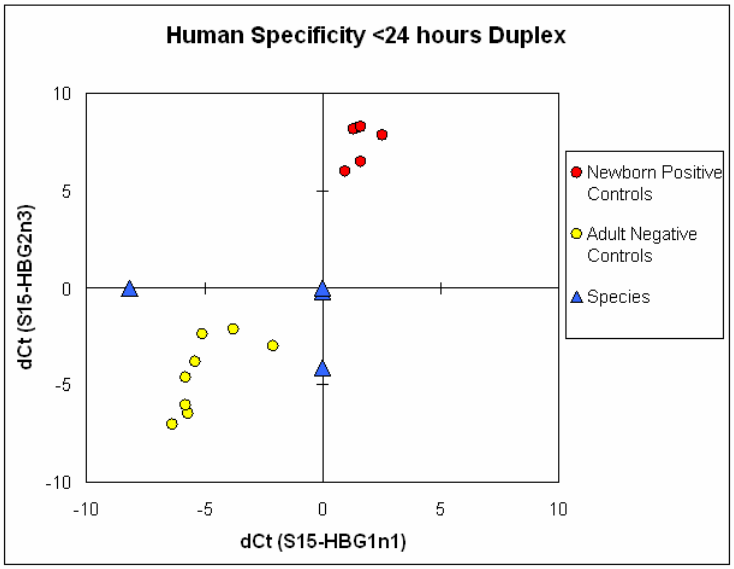
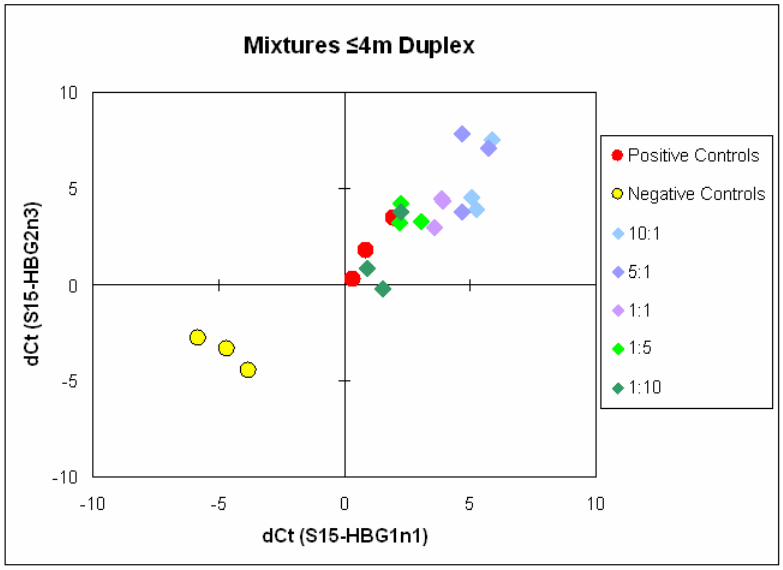


Figure 35: Human Specificity for the qPCR Newborn Duplexes

A



B

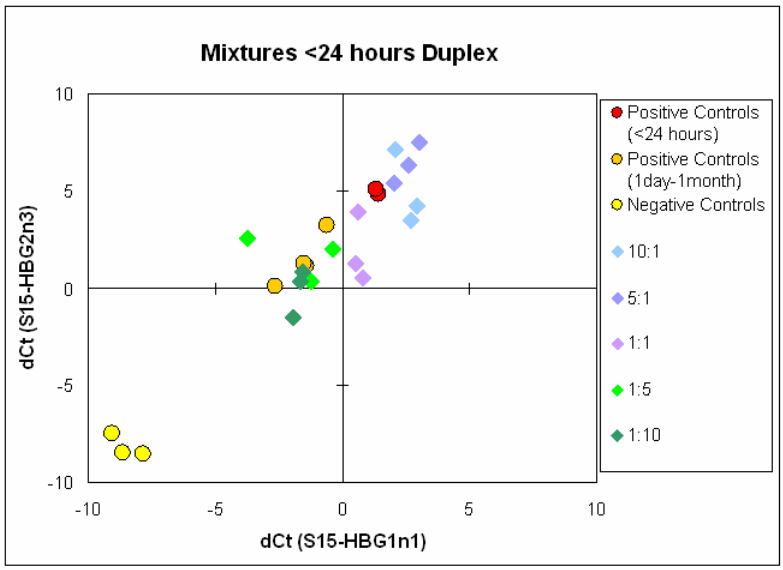


Figure 36: Mixture Study for qPCR Newborn Duplexes.

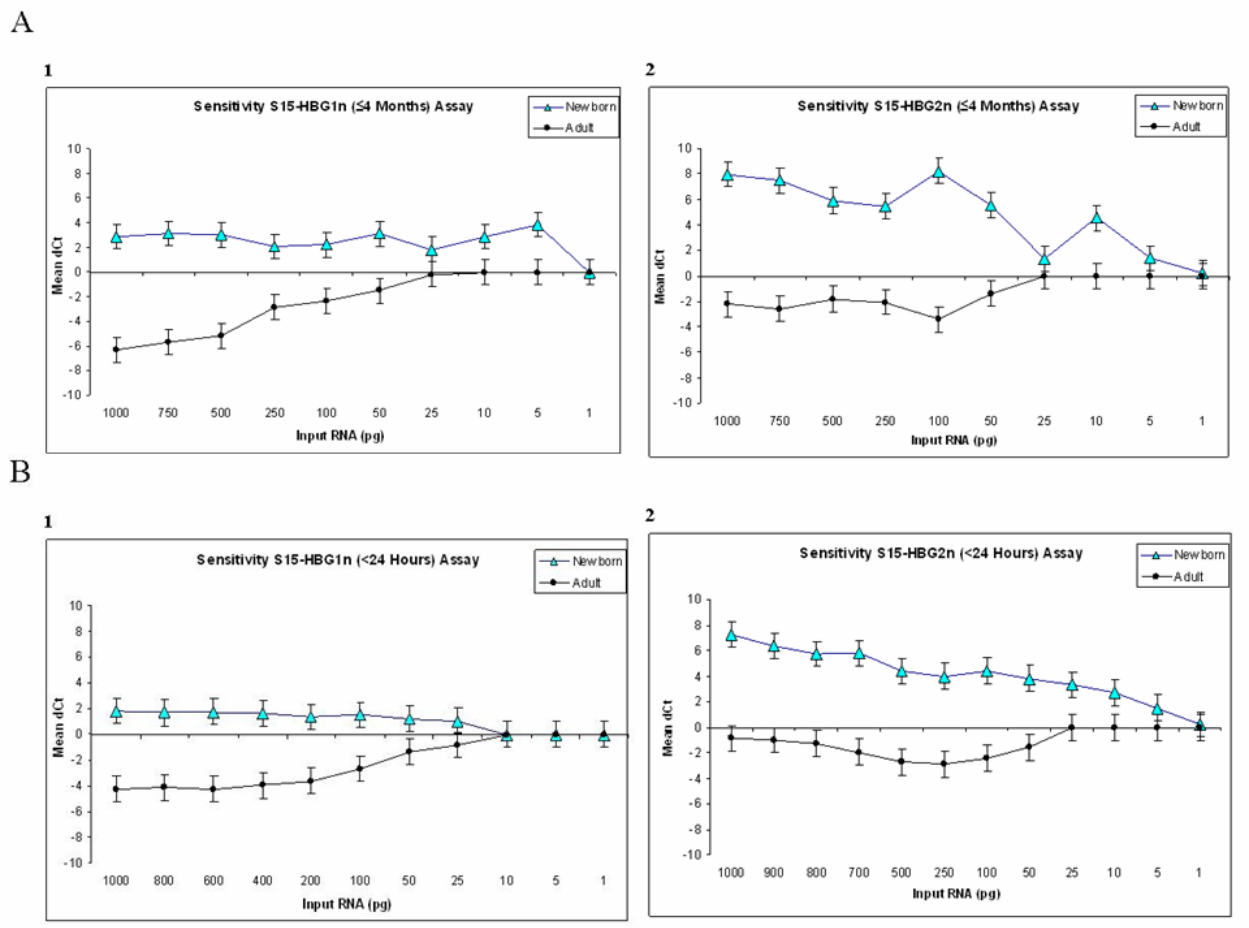


Figure 37: Sensitivity of the HBG1n1 and HBG2n3 qRT-PCR Assay.

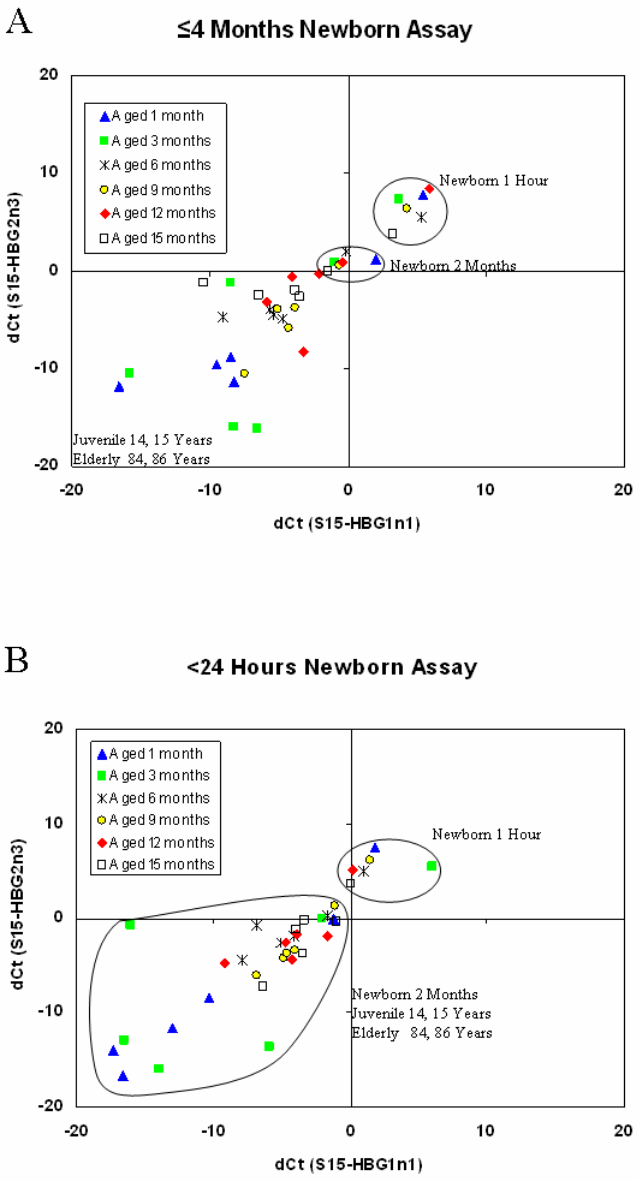


Figure 38: Temporal Stability of the HBG1n1 and HBG2n3 Transcripts in Bloodstains.

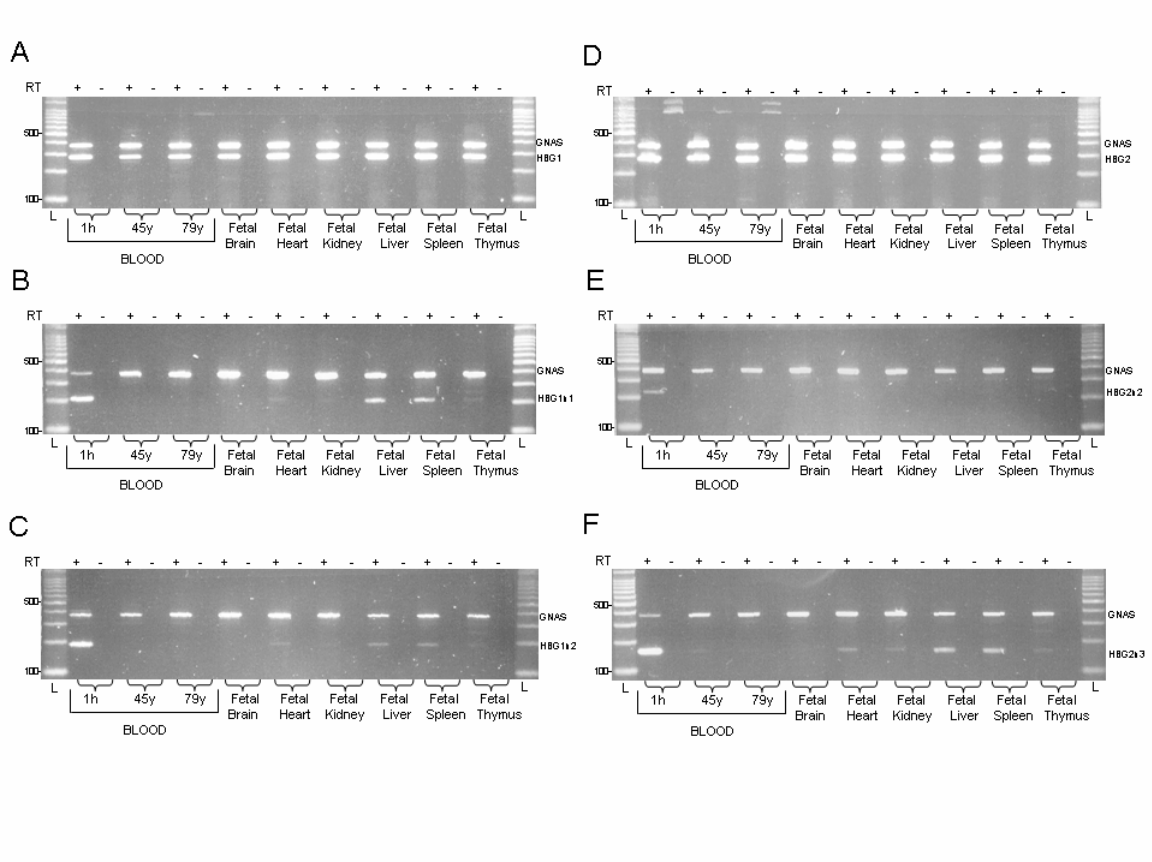


Figure 39: Hemoglobin Expression Patterns in Fetal Tissues.

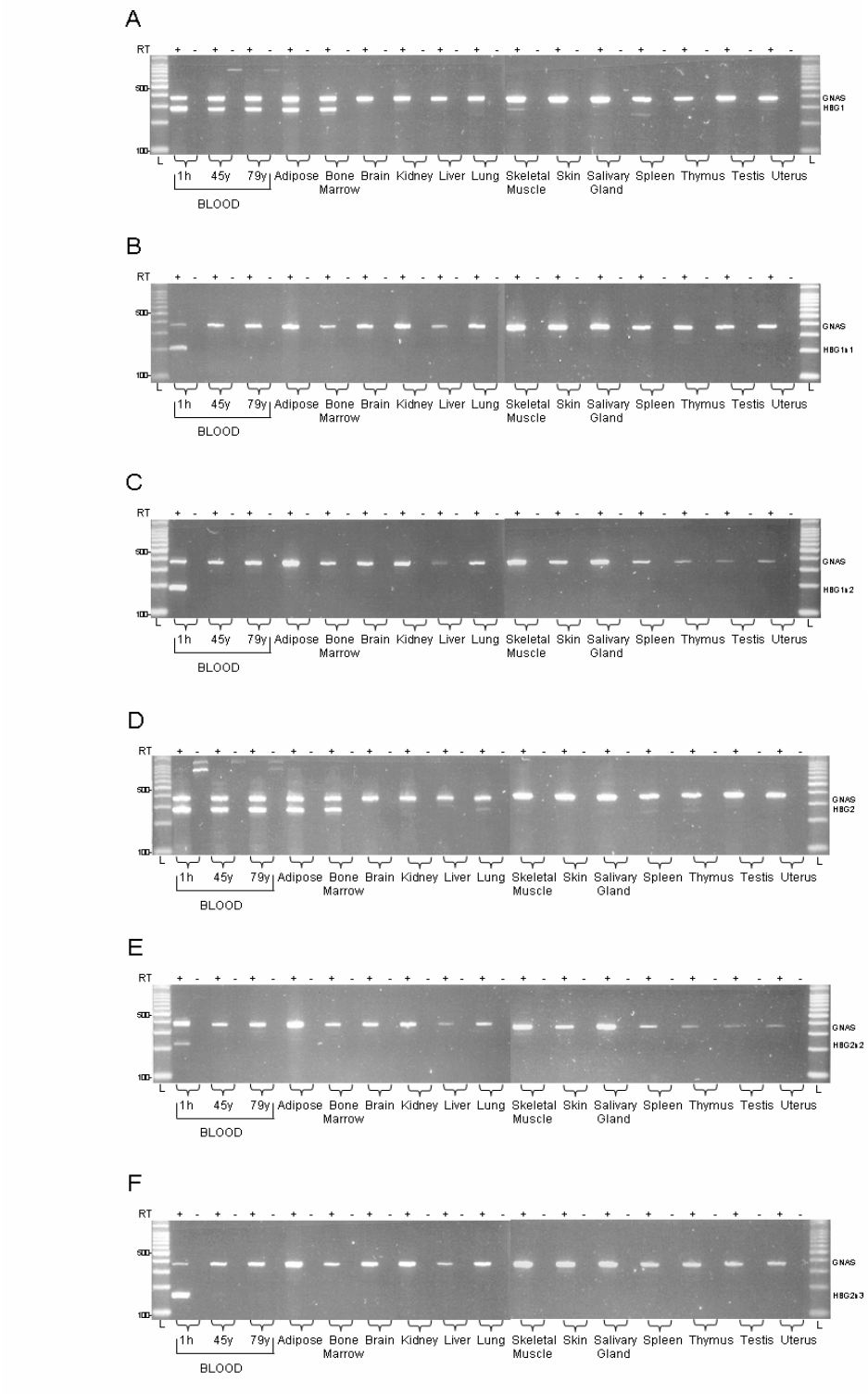


Figure 40: Hemoglobin Expression Patterns in Adult Tissues.

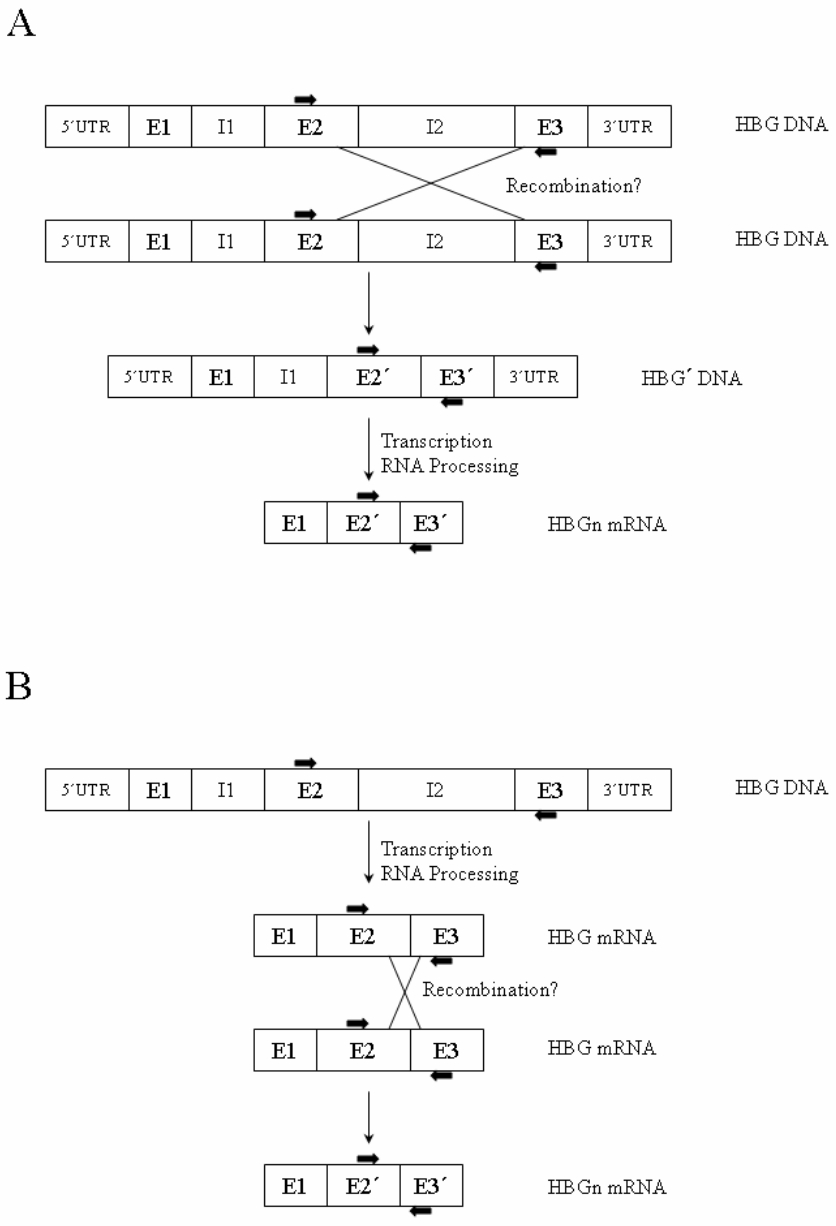


Figure 41: Postulated Rearrangement Mechanisms for the Formation of the Novel Newborn Specific Gamma Hemoglobin Transcripts.

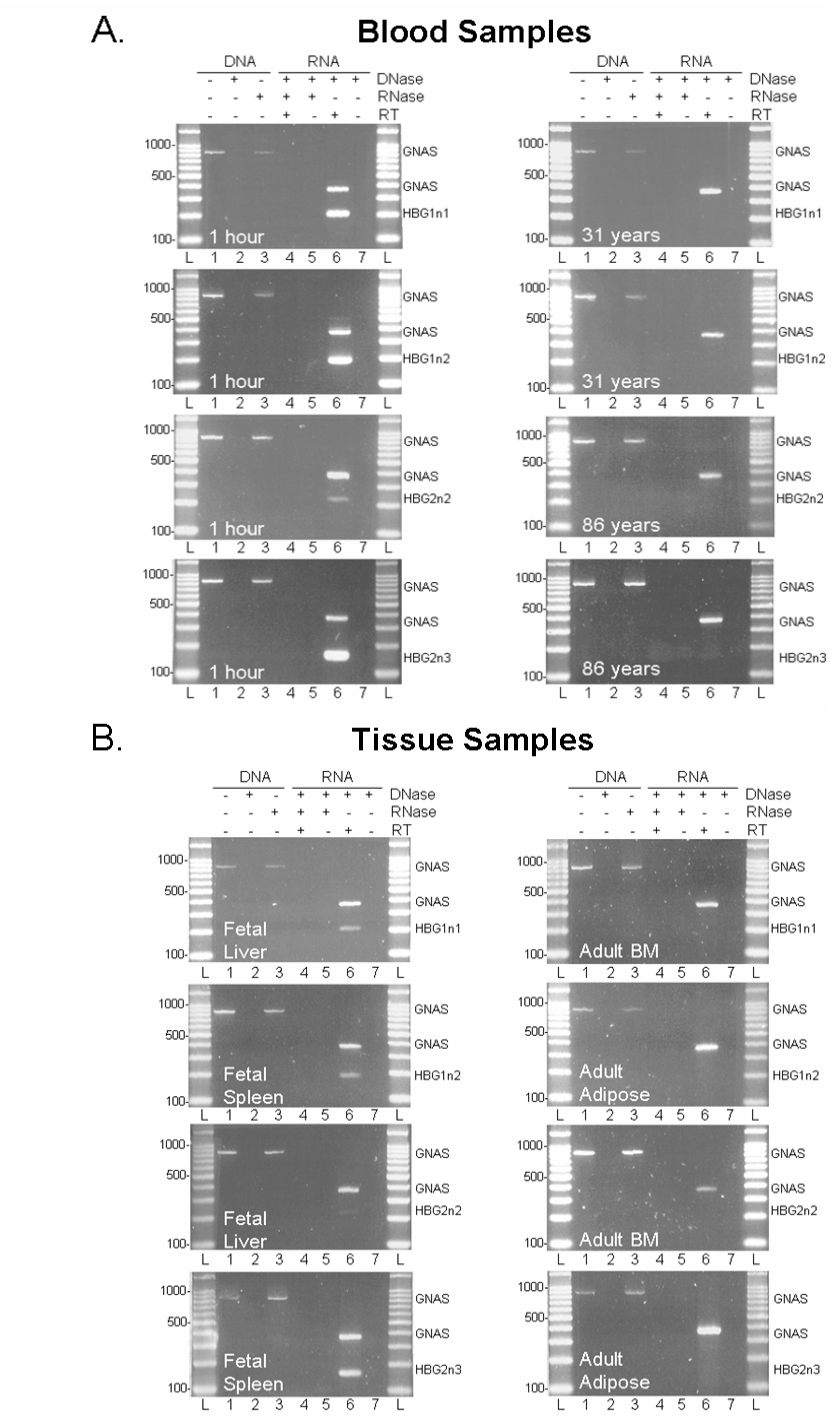


Figure 42: Newborn Hemoglobin Transcripts were Derived from an RNA Based Rearrangement Event.

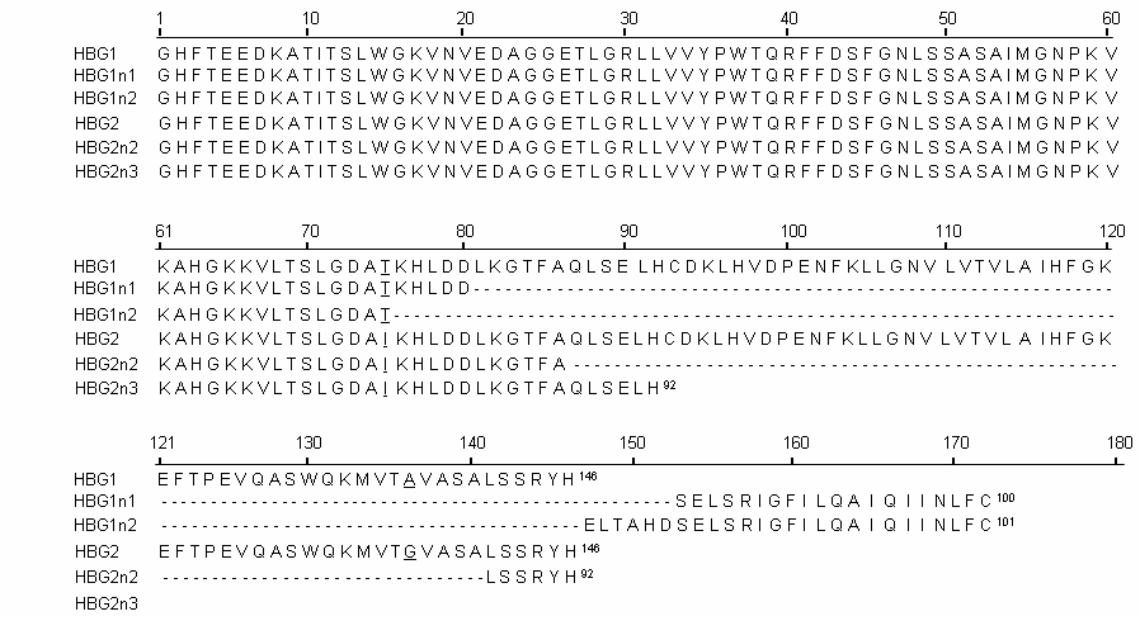


Figure 43: Alignment of the Newborn and Standard Hemoglobin Proteins.

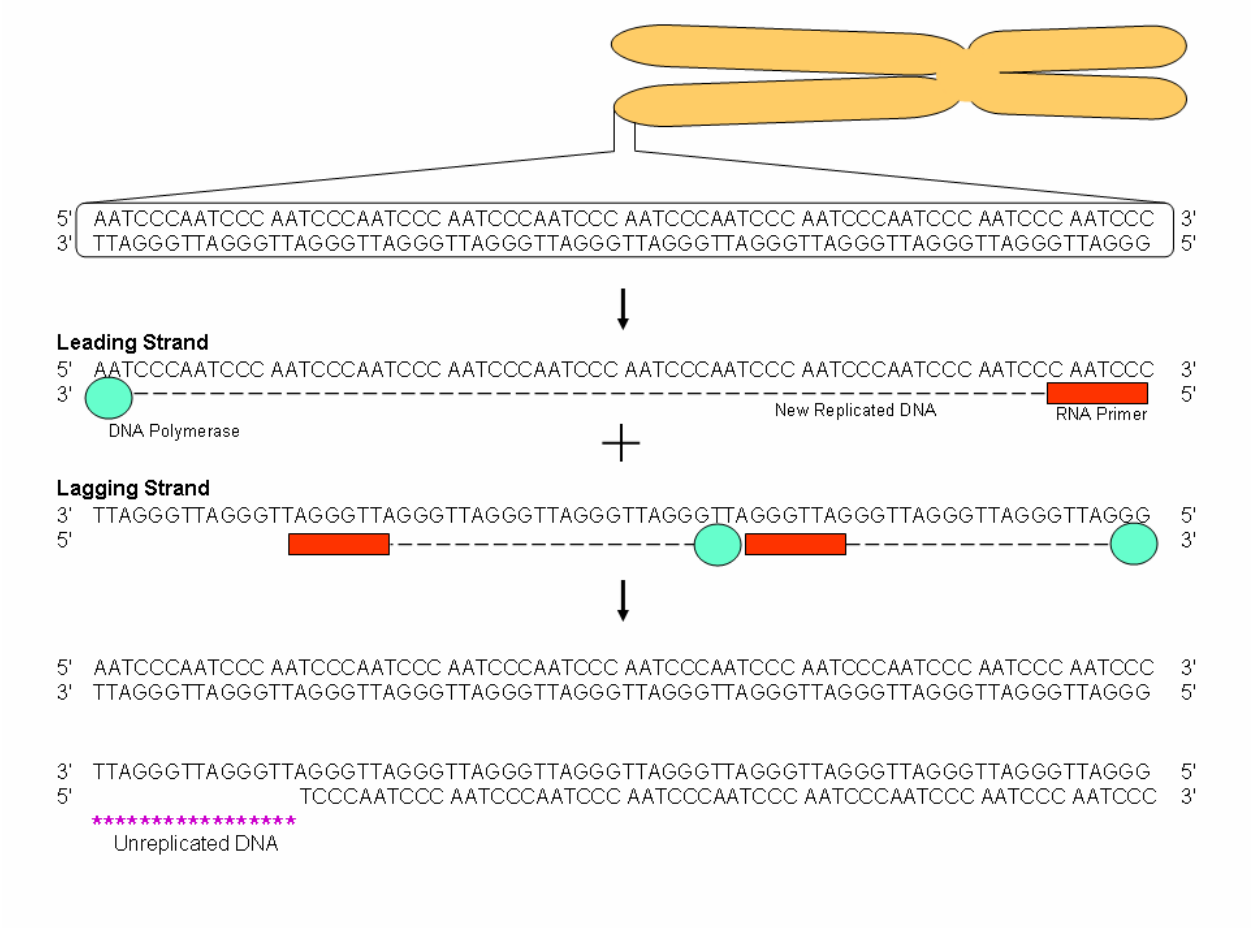


Figure 44: "End Replication Problem" of Telomeres.

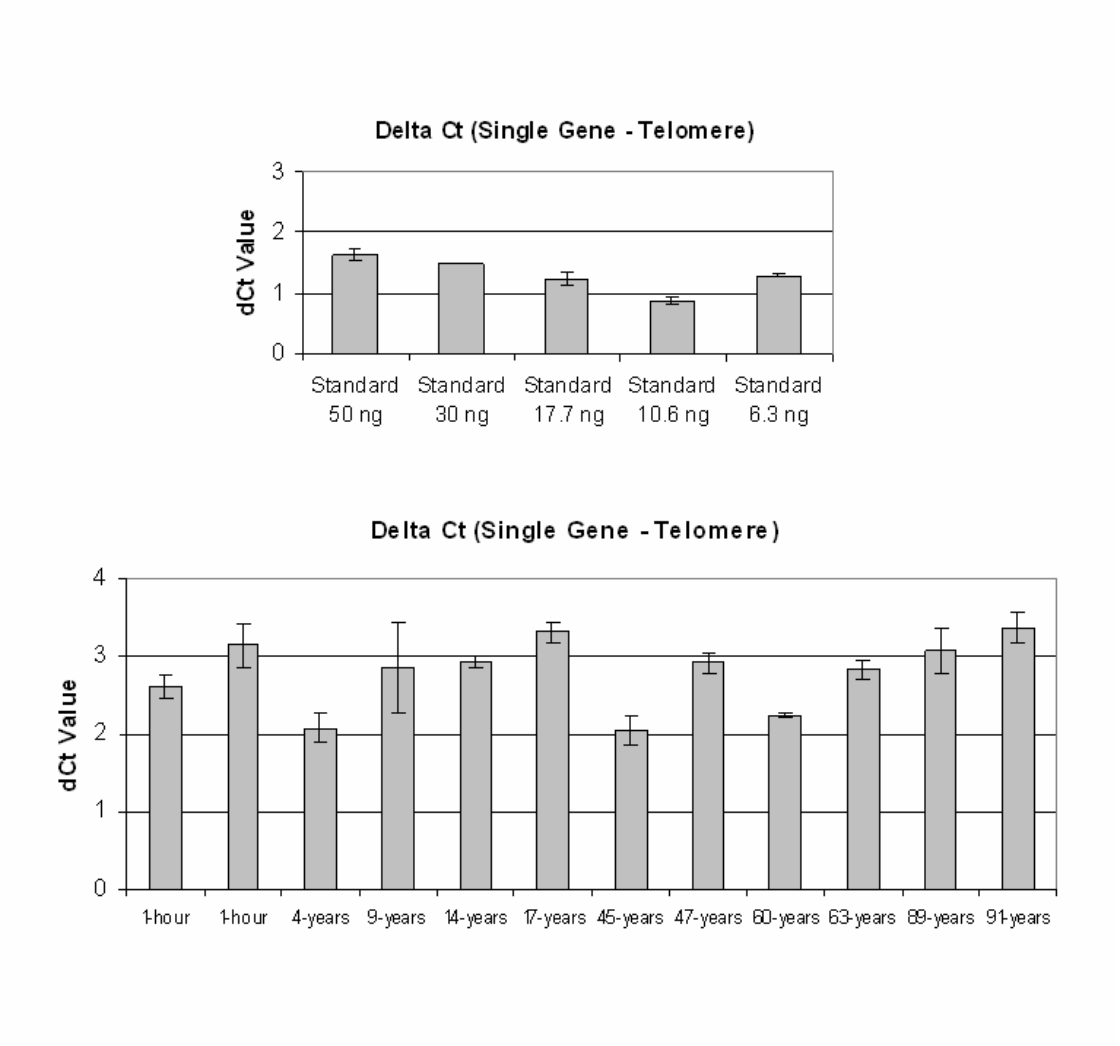


Figure 45: Telomere Delta Cycle Threshold Determination by Real-time PCR.

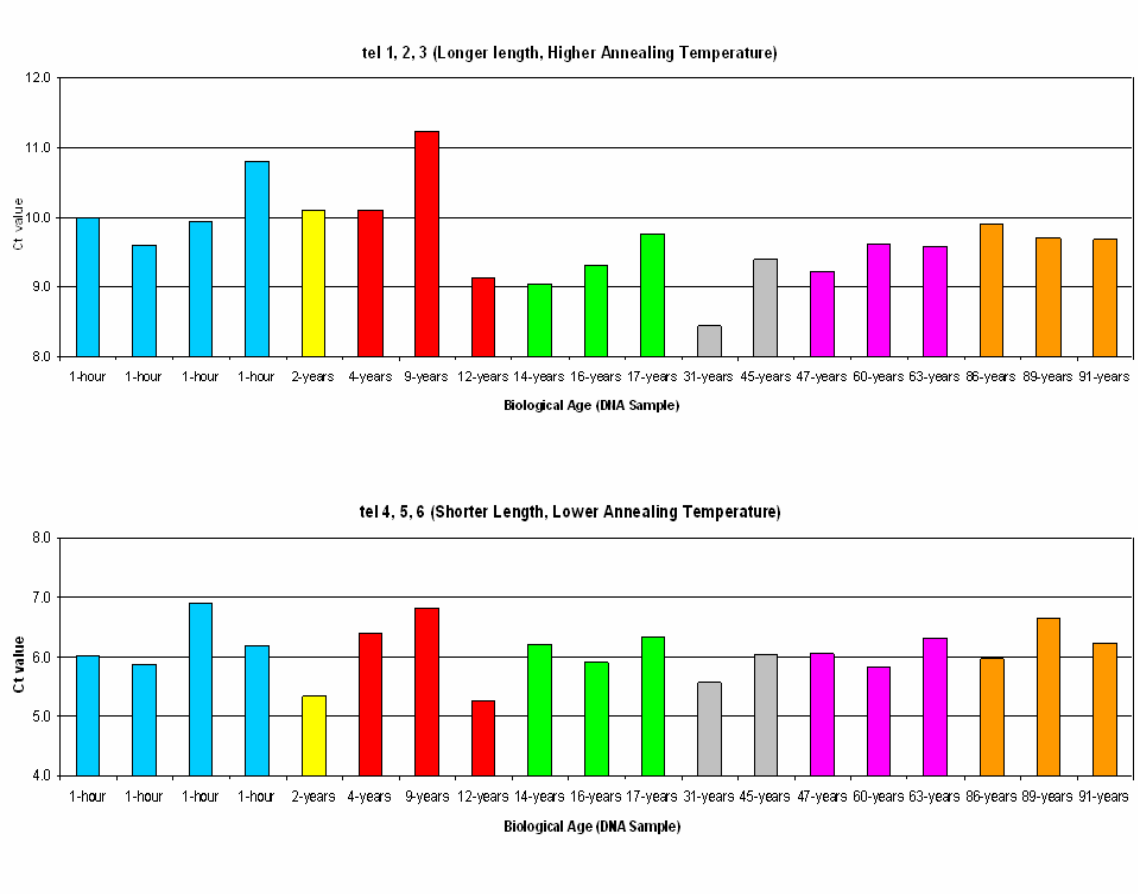


Figure 46: Quantitative Amplification of Telomeres using TaqMan Real-time PCR.

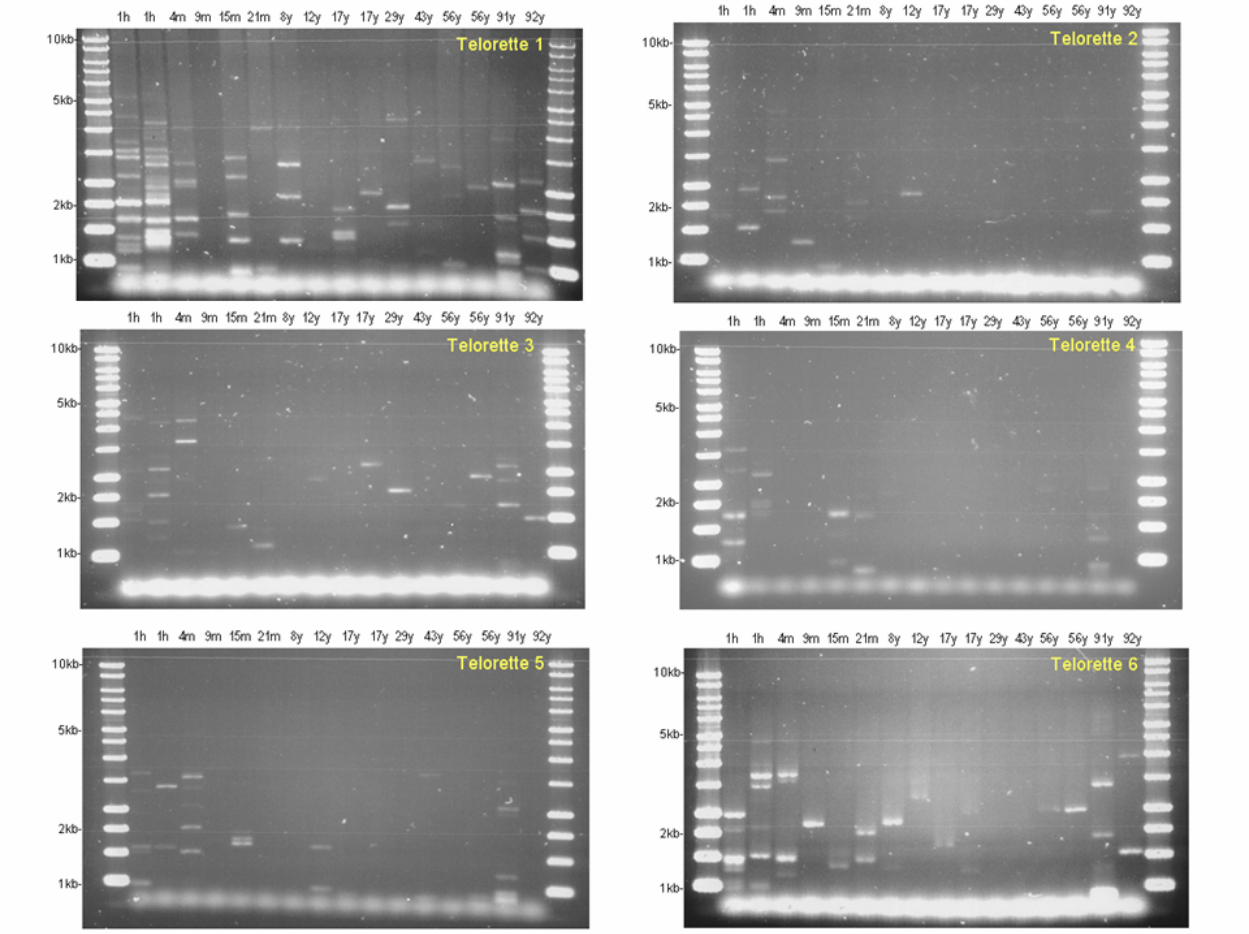


Figure 47: STELA Telomere Amplification.

APPENDIX B: TABLES

Table 1: Extracted RNA Samples for mRNA Profiling Analysis.

Age Group	Biological Age	Total Samples #	%
Newborns	1 hour - 3 months	18	12.24
Infants	4 months - 9 months	13	8.84
Toddlers	10 months - 3 years	12	8.16
Children	4 years - 12 years	13	8.84
Juveniles	13 years - 18 years	15	10.20
Adults	19 years - 45 years	30	20.41
Middle-age	46 years - 65 years	21	14.29
Elderly	66 years - 102 years	25	17.01
Total		147	100.00

Table 2: Summary of Results from RT-PCR mRNA Profiling Analysis.

Target Age Group	Source	Candidates Tested	Accepted Candidates #	Accepted Candidates %	Rejected Candidates #	Rejected Candidates %
Newborns	Affymetrix	20	2	10.00	18	90.00
	Literature	16	7	43.75	9	56.25
Juveniles	Affymetrix	63	2	3.17	61	96.83
	Literature	43	5	11.63	38	88.37
Adults	Affymetrix	0	0	0.00	0	0.00
	Literature	6	0	0.00	6	100.00
Elderly	Affymetrix	28	2	7.14	26	92.86
	Literature	143	8	5.59	135	94.41
Totals		319	26	8.15	293	91.85

Table 3: Summary and Explanation of Rejected Candidates from RT-PCR Analysis.

A

Target Age Group	Total Candidates Tested	Number Affymetrix Rejected Candidates	Rejected Explaination							
			No mRNA Detected		Same Size mRNA and DNA		mRNA Expressed in All Ages		mRNA Expressed Sporadically	
			#	%	#	%	#	%	#	%
Newborns	20	18	1	5.6	11	61.1	6	33.3	0	0.0
Juveniles	63	61	17	27.9	11	18.0	22	36.1	11	18.0
Elderly	28	26	1	3.8	5	19.2	9	34.6	11	42.3
Totals	111	105	19	6.0	27	8.5	37	11.6	22	6.9

B

Target Age Group	Total Candidates Tested	Number Literature Rejected Candidates	Rejected Explaination							
			No mRNA Detected		Same Size mRNA and DNA		mRNA Expressed in All Ages		mRNA Expressed Sporadically	
			#	%	#	%	#	%	#	%
Newborns	16	9	3	33.3	0	0.0	3	33.3	3	33.3
Juveniles	43	38	19	50.0	3	7.9	3	7.9	13	34.2
Adults	6	6	0	0.0	0	0.0	4	66.7	2	33.3
Elderly	143	135	27	20.0	0	0.0	81	60.0	27	20.0
Totals	208	188	49	15.4	3	0.9	91	28.5	45	14.1

Table 4: Real-Time PCR Cycle Threshold Values for Candidate Gene Biological Age Testing.

CDC2-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	37.624
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	36.091
Newborn -RT				Undet
Infant +RT	M	8m	0.667	35.623
Infant -RT				Undet
Toddler +RT	M	2y	2.000	36.085
Toddler -RT				Undet
Child +RT	M	4y	4.000	35.065
Child -RT				Undet
Child +RT	M	5y	5.000	34.271
Child -RT				Undet

CDC2-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Child +RT	M	9y	9.000	38.501
Child -RT				Undet
Juvenile +RT	M	15y	15.000	30.782
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	36.003
Adult -RT				Undet
Middle-Age +RT	F	58y	58.000	38.134
Middle-Age -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

POLQ-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	3m	0.250	35.634
Newborn -RT				Undet
Toddler +RT	M	2y	2.000	36.024
Toddler -RT				Undet
Child +RT	M	4y	4.000	34.012
Child -RT				Undet
Juvenile +RT	M	13y	13.000	36.736
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	35.004
Adult -RT				Undet
Adult +RT	F	40y	40.000	37.099
Adult -RT				Undet

POLQ-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Middle-Age +RT	M	57y	57.000	36.809
Middle-Age -RT				Undet
Elderly +RT	F	66y	66.000	35.446
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	35.209
Elderly -RT				Undet
Elderly +RT	F	89y	89.000	36.648
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

SRC-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	38.635
Newborn -RT				Undet
Newborn +RT	F	8d	0.022	34.962
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	36.649
Newborn -RT				Undet
Child +RT	M	4y	4.000	35.684
Child -RT				Undet
Juvenile +RT	F	16y	16.000	36.712
Juvenile -RT				3.000
Adult +RT	F	31y	31.000	37.135
Adult -RT				Undet

SRC-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Adult +RT	F	40y	40.000	36.666
Adult -RT				Undet
Middle-Age +RT	M	57y	57.000	36.769
Middle-Age -RT				Undet
Elderly +RT	F	66y	66.000	35.986
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	37.333
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

LOH11CR2A-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	38.690
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	36.469
Newborn -RT				Undet
Infant +RT	M	8m	0.667	40.000
Infant -RT				Undet
Toddler +RT	M	18m	1.500	34.846
Toddler -RT				Undet
Toddler +RT	F	3y	3.000	34.157
Toddler -RT				Undet
Child +RT	M	5y	5.000	34.840
Child -RT				Undet
Child +RT	F	12y	12.000	36.659
Child -RT				Undet
Juvenile +RT	M	14y	14.000	36.144
Juvenile -RT				Undet
Juvenile +RT	F	16y	16.000	38.224
Juvenile -RT				Undet

LOH11CR2A-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Adult +RT	F	38y	38.000	38.227
Adult -RT				Undet
Middle-Age +RT	F	53y	53.000	40.000
Middle-Age -RT				Undet
Middle-Age +RT	F	63y	63.000	34.774
Middle-Age -RT				Undet
Elderly +RT	F	71y	71.000	40.000
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	34.505
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	35.656
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

ASL-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.00274	36.386
Newborn -RT				Undet
Newborn +RT	F	17d	0.04658	34.818
Newborn -RT				Undet
Infant +RT	M	4m	0.33333	35.006
Infant -RT				Undet
Toddler +RT	M	10m	0.83333	33.996
Toddler -RT				Undet
Toddler +RT	F	3y	3	33.986
Toddler -RT				Undet
Child +RT	M	6y	6	34.661
Child -RT				Undet
Juvenile +RT	M	14y	14	34.601
Juvenile -RT				Undet
Juvenile +RT	F	18y	18	35.188
Juvenile -RT				Undet
Adult +RT	M	29y	29	34.702
Adult -RT				Undet
Adult +RT	M	36y	36	36.078
Adult -RT				Undet
Middle-Age +RT	F	47y	47	36.137
Middle-Age -RT				Undet
Middle-Age +RT	M	59y	59	36.028
Middle-Age -RT				Undet
Middle-Age +RT	M	65y	65	38.008
Middle-Age -RT				Undet

ASL-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Elderly +RT	F	68y	68	40.244
Elderly -RT				Undet
Elderly +RT	M	71y	71	37.519
Elderly -RT				Undet
Elderly +RT	M	74y	74	39.472
Elderly -RT				Undet
Elderly +RT	F	76y	76	38.187
Elderly -RT				Undet
Elderly +RT	F	81y	81	36.569
Elderly -RT				Undet
Elderly +RT	F	84y	84	34.954
Elderly -RT				Undet
Elderly +RT	M	89y	89	35.069
Elderly -RT				Undet
Elderly +RT	F	89y	89	36.139
Elderly -RT				Undet
Elderly +RT	F	91y	91	33.912
Elderly -RT				Undet
Elderly +RT	M	92y	92	35.481
Elderly -RT				Undet
Elderly +RT	F	102y	102	32.619
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

FLJ20344a-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	38.105
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	40.000
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	38.601
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	39.272
Newborn -RT				Undet
Infant +RT	M	8m	0.667	40.000
Infant -RT				Undet
Toddler +RT	M	18m	1.500	38.428
Toddler -RT				Undet
Toddler +RT	F	3y	3.000	37.314
Toddler -RT				Undet
Child +RT	M	9y	9.000	40.000
Child -RT				Undet
Juvenile +RT	F	15y	15.000	40.000
Juvenile -RT				Undet

FLJ20344a-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Adult +RT	M	24y	24.000	40.000
Adult -RT				Undet
Adult +RT	M	45y	45.000	38.559
Adult -RT				Undet
Middle-Age +RT	F	60y	60.000	40.000
Middle-Age -RT				Undet
Elderly +RT	M	72y	72.000	40.000
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	40.000
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	40.000
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

LOC151194-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	35.389
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	33.941
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	36.287
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	33.668
Newborn -RT				Undet
Infant +RT	M	8m	0.667	36.086
Infant -RT				Undet
Toddler +RT	M	18m	1.500	33.588
Toddler -RT				Undet
Toddler +RT	F	3y	3.000	32.604
Toddler -RT				Undet
Child +RT	M	9y	9.000	37.770
Child -RT				Undet
Juvenile +RT	F	15y	15.000	33.916
Juvenile -RT				Undet

LOC151194-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Adult +RT	M	24y	24.000	35.985
Adult -RT				Undet
Adult +RT	M	45y	45.000	34.896
Adult -RT				Undet
Middle-Age +RT	F	60y	60.000	34.940
Middle-Age -RT				Undet
Elderly +RT	M	72y	72.000	36.263
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	35.814
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	35.402
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

SPTRX-1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	40.000
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	36.830
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	40.000
Newborn -RT				Undet
Infant +RT	F	5m	0.417	38.430
Infant -RT				Undet
Infant +RT	F	7m	0.583	40.000
Infant -RT				Undet
Toddler +RT	M	14m	1.167	40.000
Toddler -RT				Undet
Toddler +RT	F	19m	1.583	40.000
Toddler -RT				Undet
Toddler +RT	M	2y	2.000	38.097
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	39.197
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	40.000
Toddler -RT				Undet
Child +RT	F	4y	4.000	40.000
Child -RT				Undet
Child +RT	F	6y	6.000	38.382
Child -RT				Undet

SPTRX-1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Child +RT	M	12y	12.000	37.864
Child -RT				Undet
Juvenile +RT	M	14y	14.000	40.000
Juvenile -RT				Undet
Juvenile +RT	F	16y	16.000	40.000
Juvenile -RT				Undet
Juvenile +RT	F	18y	18.000	36.509
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	38.579
Adult -RT				Undet
Adult +RT	F	40y	40.000	36.566
Adult -RT				Undet
Middle-Age +RT	F	53y	53.000	40.000
Middle-Age -RT				Undet
Elderly +RT	M	66y	66.000	40.000
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	37.852
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	38.282
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

SPTRX-2-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	40.000
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	33.052
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	35.297
Newborn -RT				Undet
Infant +RT	F	5m	0.417	37.869
Infant -RT				Undet
Infant +RT	F	7m	0.583	36.700
Infant -RT				Undet
Toddler +RT	M	14m	1.167	38.775
Toddler -RT				Undet
Toddler +RT	F	19m	1.583	35.808
Toddler -RT				Undet
Toddler +RT	M	2y	2.000	35.584
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	36.214
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	35.656
Toddler -RT				Undet
Child +RT	F	4y	4.000	40.000
Child -RT				Undet
Child +RT	F	6y	6.000	34.290
Child -RT				Undet

SPTRX-2-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Child +RT	M	12y	12.000	35.984
Child -RT				Undet
Juvenile +RT	M	14y	14.000	37.840
Juvenile -RT				Undet
Juvenile +RT	F	16y	16.000	37.005
Juvenile -RT				Undet
Juvenile +RT	F	18y	18.000	36.661
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	35.858
Adult -RT				Undet
Adult +RT	F	40y	40.000	35.364
Adult -RT				Undet
Middle-Age +RT	F	53y	53.000	40.000
Middle-Age -RT				Undet
Elderly +RT	M	66y	66.000	40.000
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	35.581
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	36.067
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

POX-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	35.933
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	34.208
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	36.860
Newborn -RT				Undet
Infant +RT	F	5m	0.417	37.395
Infant -RT				Undet
Infant +RT	F	7m	0.583	36.486
Infant -RT				Undet
Toddler +RT	M	14m	1.167	39.953
Toddler -RT				Undet
Toddler +RT	F	19m	1.583	37.035
Toddler -RT				Undet
Toddler +RT	M	2y	2.000	38.196
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	36.802
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	38.285
Toddler -RT				Undet
Child +RT	F	4y	4.000	40.000
Child -RT				Undet
Child +RT	F	6y	6.000	38.750
Child -RT				Undet

PPOX-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Child +RT	M	12y	12.000	38.058
Child -RT				Undet
Juvenile +RT	M	14y	14.000	39.812
Juvenile -RT				Undet
Juvenile +RT	F	16y	16.000	40
Juvenile -RT				Undet
Juvenile +RT	F	18y	18.000	37.258
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	36.979
Adult -RT				Undet
Adult +RT	F	40y	40.000	37.760
Adult -RT				Undet
Middle-Age +RT	F	53y	53.000	40.000
Middle-Age -RT				Undet
Elderly +RT	M	66y	66.000	38.938
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	37.102
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	36.681
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

TBC1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	40.000
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	38.617
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	38.384
Newborn -RT				Undet
Infant +RT	F	5m	0.417	37.573
Infant -RT				Undet
Infant +RT	F	7m	0.583	35.573
Infant -RT				Undet
Toddler +RT	M	14m	1.167	39.714
Toddler -RT				Undet
Toddler +RT	F	19m	1.583	38.165
Toddler -RT				Undet
Toddler +RT	M	2y	2.000	39.001
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	39.849
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	39.397
Toddler -RT				Undet
Child +RT	F	4y	4.000	36.400
Child -RT				Undet
Child +RT	F	6y	6.000	36.597
Child -RT				Undet

TBC1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Child +RT	M	12y	12.000	39.450
Child -RT				Undet
Juvenile +RT	M	14y	14.000	40.000
Juvenile -RT				Undet
Juvenile +RT	F	16y	16.000	40.000
Juvenile -RT				Undet
Juvenile +RT	F	18y	18.000	36.475
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	34.083
Adult -RT				Undet
Adult +RT	F	40y	40.000	37.052
Adult -RT				Undet
Middle-Age +RT	F	53y	53.000	40.000
Middle-Age -RT				Undet
Elderly +RT	M	66y	66.000	40.000
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	38.653
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	38.521
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

TEKT2-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	40.000
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	39.304
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	40.000
Newborn -RT				Undet
Infant +RT	F	5m	0.417	36.439
Infant -RT				Undet
Infant +RT	F	7m	0.583	40.000
Infant -RT				Undet
Toddler +RT	M	14m	1.167	40.000
Toddler -RT				Undet
Toddler +RT	F	19m	1.583	39.248
Toddler -RT				Undet
Toddler +RT	M	2y	2.000	39.447
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	38.297
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	40.000
Toddler -RT				Undet
Child +RT	F	4y	4.000	40.000
Child -RT				Undet
Child +RT	F	6y	6.000	39.312
Child -RT				Undet

TEKT2-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Child +RT	M	12y	12.000	40.000
Child -RT				Undet
Juvenile +RT	M	14y	14.000	39.077
Juvenile -RT				Undet
Juvenile +RT	F	16y	16.000	40.000
Juvenile -RT				Undet
Juvenile +RT	F	18y	18.000	40.000
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	40.000
Adult -RT				Undet
Adult +RT	F	40y	40.000	40.000
Adult -RT				Undet
Middle-Age +RT	F	53y	53.000	40.000
Middle-Age -RT				Undet
Elderly +RT	M	66y	66.000	40.000
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	39.257
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	40.000
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

PRL-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	40.000
Newborn -RT				Undet
Newborn +RT	F	1m	0.083	35.424
Newborn -RT				Undet
Newborn +RT	M	3m	0.250	40.000
Newborn -RT				Undet
Infant +RT	F	5m	0.417	38.798
Infant -RT				Undet
Infant +RT	F	7m	0.583	35.407
Infant -RT				Undet
Toddler +RT	M	14m	1.167	38.126
Toddler -RT				Undet
Toddler +RT	F	19m	1.583	38.244
Toddler -RT				Undet
Toddler +RT	M	2y	2.000	37.261
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	40.000
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	40.000
Toddler -RT				Undet
Child +RT	F	4y	4.000	40.000
Child -RT				Undet
Child +RT	F	6y	6.000	38.700
Child -RT				Undet

PRL-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Child +RT	M	12y	12.000	38.363
Child -RT				Undet
Juvenile +RT	M	14y	14.000	40.000
Juvenile -RT				Undet
Juvenile +RT	F	16y	16.000	40.000
Juvenile -RT				Undet
Juvenile +RT	F	18y	18.000	38.633
Juvenile -RT				Undet
Adult +RT	F	31y	31.000	40.000
Adult -RT				Undet
Adult +RT	F	40y	40.000	40.000
Adult -RT				Undet
Middle-Age +RT	F	53y	53.000	40.000
Middle-Age -RT				Undet
Elderly +RT	M	66y	66.000	40.000
Elderly -RT				Undet
Elderly +RT	M	79y	79.000	40.000
Elderly -RT				Undet
Elderly +RT	M	89y	89.000	40.000
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

Table 5: Real-Time PCR First Round Candidate Results.

AGGF1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	37.910
Newborn -RT				Undet
Newborn +RT	F	8d	0.022	34.738
Newborn -RT				Undet
Newborn +RT	F	3m	0.250	35.838
Newborn -RT				Undet
Infant +RT	F	6m	0.500	36.086
Infant -RT				Undet
Toddler +RT	M	10m	0.833	37.095
Toddler -RT				Undet
Toddler +RT	F	21m	1.750	35.942
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	36.747
Toddler -RT				Undet
Child +RT	M	5y	5.000	35.635
Child -RT				Undet
Child +RT	F	9y	9.000	38.361
Child -RT				Undet
Child +RT	M	12y	12.000	37.210
Child -RT				Undet
Juvenile +RT	M	13y	13.000	36.632
Juvenile -RT				Undet
Juvenile +RT	M	13y	13.000	36.616
Juvenile -RT				Undet
Juvenile +RT	F	14y	14.000	35.855
Juvenile -RT				Undet
Juvenile +RT	M	14y	14.000	37.084
Juvenile -RT				Undet
Juvenile +RT	F	15y	15.000	36.552
Juvenile -RT				Undet
Juvenile +RT	M	16y	16.000	36.262
Juvenile -RT				Undet

AGGF1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	M	17y	17.000	40.226
Juvenile -RT				Undet
Juvenile +RT	M	18y	18.000	38.078
Juvenile -RT				Undet
Adult +RT	M	23y	23.000	40.220
Adult -RT				Undet
Adult +RT	F	31y	31.000	38.075
Adult -RT				Undet
Adult +RT	F	38y	38.000	37.850
Adult -RT				Undet
Middle-Age +RT	F	47y	47.000	36.699
Middle-Age -RT				Undet
Middle-Age +RT	F	56y	56.000	39.024
Middle-Age -RT				Undet
Middle-Age +RT	M	63y	63.000	40.325
Middle-Age -RT				Undet
Elderly +RT	F	69y	69.000	39.531
Elderly -RT				Undet
Elderly +RT	M	74y	74.000	38.497
Elderly -RT				Undet
Elderly +RT	M	83y	83.000	41.200
Elderly -RT				Undet
Elderly +RT	F	89y	89.000	37.350
Elderly -RT				Undet
Elderly +RT	M	92y	92.000	38.659
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

MAD1L1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	33.859
Newborn -RT				Undet
Newborn +RT	M	1m	0.083	32.639
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	32.352
Newborn -RT				Undet
Infant +RT	M	5m	0.417	32.101
Infant -RT				Undet
Infant +RT	F	9m	0.750	31.655
Infant -RT				Undet
Toddler +RT	M	15m	1.250	32.464
Toddler -RT				Undet
Toddler +RT	F	21m	1.750	31.636
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	34.260
Toddler -RT				Undet
Child +RT	M	4y	4.000	31.075
Child -RT				Undet
Child +RT	F	4y	4.000	33.428
Child -RT				Undet
Child +RT	M	6y	6.000	32.105
Child -RT				Undet
Child +RT	F	9y	9.000	34.052
Child -RT				Undet
Child +RT	M	12y	12.000	32.586
Child -RT				Undet
Juvenile +RT	M	13y	13.000	34.398
Juvenile -RT				Undet
Juvenile +RT	M	14y	14.000	32.349
Juvenile -RT				Undet
Juvenile +RT	M	15y	15.000	32.451
Juvenile -RT				Undet

MAD1L1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	M	17y	17.000	38.530
Juvenile -RT				Undet
Adult +RT	M	19y	19.000	32.931
Adult -RT				Undet
Adult +RT	M	24y	24.000	32.286
Adult -RT				Undet
Adult +RT	F	31y	31.000	32.262
Adult -RT				Undet
Adult +RT	M	38y	38.000	32.485
Adult -RT				Undet
Adult +RT	M	45y	45.000	32.100
Adult -RT				Undet
Middle-Age +RT	M	51y	51.000	32.777
Middle-Age -RT				Undet
Middle-Age +RT	F	58y	58.000	33.705
Middle-Age -RT				Undet
Elderly +RT	M	65y	65.000	33.602
Elderly -RT				Undet
Elderly +RT	F	71y	71.000	36.551
Elderly -RT				Undet
Elderly +RT	F	76y	76.000	33.497
Elderly -RT				Undet
Elderly +RT	M	83y	83.000	34.930
Elderly -RT				Undet
Elderly +RT	F	91y	91.000	33.187
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

PDCD6-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	32.705
Newborn -RT				Undet
Newborn +RT	M	13d	0.036	32.089
Newborn -RT				Undet
Newborn +RT	M	1m	0.083	29.960
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	31.676
Newborn -RT				Undet
Infant +RT	M	5m	0.417	31.088
Infant -RT				Undet
Infant +RT	F	9m	0.750	30.036
Infant -RT				Undet
Toddler +RT	M	15m	1.250	32.980
Toddler -RT				Undet
Toddler +RT	M	2.8y	2.800	33.405
Toddler -RT				Undet
Child +RT	M	4y	4.000	29.344
Child -RT				Undet
Child +RT	M	8y	8.000	31.188
Child -RT				Undet
Child +RT	M	12y	12.000	32.183
Child -RT				Undet
Juvenile +RT	M	15y	15.000	29.442
Juvenile -RT				Undet
Adult +RT	M	19y	19.000	30.664
Adult -RT				Undet
Adult +RT	M	24y	24.000	30.230
Adult -RT				Undet
Adult +RT	M	29y	29.000	30.743
Adult -RT				Undet

PDCD6-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Adult +RT	F	35y	35.000	33.250
Adult -RT				Undet
Adult +RT	M	38y	38.000	32.048
Adult -RT				Undet
Adult +RT	F	43y	43.000	33.073
Adult -RT				Undet
Middle-Age +RT	M	47y	47.000	32.093
Middle-Age -RT				Undet
Middle-Age +RT	F	53y	53.000	38.182
Middle-Age -RT				Undet
Middle-Age +RT	M	59y	59.000	33.505
Middle-Age -RT				Undet
Elderly +RT	M	65y	65.000	32.479
Elderly -RT				Undet
Elderly +RT	F	68y	68.000	31.293
Elderly -RT				Undet
Elderly +RT	F	71y	71.000	31.672
Elderly -RT				Undet
Elderly +RT	F	76y	76.000	33.004
Elderly -RT				Undet
Elderly +RT	F	81y	81.000	33.258
Elderly -RT				Undet
Elderly +RT	M	86y	86.000	32.128
Elderly -RT				Undet
Elderly +RT	F	91y	91.000	29.600
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

PPARD-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	34.078
Newborn -RT				Undet
Newborn +RT	M	1m	0.083	33.044
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	32.827
Newborn -RT				Undet
Infant +RT	M	5m	0.417	31.710
Infant -RT				Undet
Infant +RT	F	9m	0.750	32.254
Infant -RT				Undet
Toddler +RT	M	15m	1.250	32.690
Toddler -RT				Undet
Toddler +RT	F	21m	1.750	32.060
Toddler -RT				Undet
Toddler +RT	M	3y	3.000	34.412
Toddler -RT				Undet
Child +RT	M	5y	5.000	31.771
Child -RT				Undet
Child +RT	F	9y	9.000	34.561
Child -RT				Undet
Child +RT	M	14y	14.000	32.793
Child -RT				Undet
Child +RT	M	19y	19.000	33.232
Child -RT				Undet
Child +RT	M	24y	24.000	32.705
Child -RT				Undet
Juvenile +RT	F	31y	31.000	32.767
Juvenile -RT				Undet

PPARD-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	F	35y	35.000	34.372
Juvenile -RT				Undet
Juvenile +RT	M	40y	40.000	33.203
Juvenile -RT				Undet
Juvenile +RT	M	45y	45.000	32.760
Juvenile -RT				Undet
Adult +RT	M	51y	51.000	33.782
Adult -RT				Undet
Adult +RT	M	56y	56.000	34.921
Adult -RT				Undet
Adult +RT	M	61y	61.000	33.688
Adult -RT				Undet
Adult +RT	M	65y	65.000	33.723
Adult -RT				Undet
Adult +RT	F	71y	71.000	36.565
Adult -RT				Undet
Middle-Age +RT	F	76y	76.000	33.450
Middle-Age -RT				Undet
Middle-Age +RT	M	83y	83.000	37.131
Middle-Age -RT				Undet
Elderly +RT	F	91y	91.000	34.315
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

Table 6: Real-Time PCR Duplex Delta Ct Results.

AGGF1-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	47.528	34.531	-12.997
Newborn +RT	1m	42.358	32.725	-9.633
Infant +RT	6m	42.544	33.715	-8.829
Toddler +RT	14m	44.931	34.623	-10.308
Child +RT	4y	45.015	36.543	-8.472
Child +RT	12y	41.466	33.554	-7.912
Juvenile +RT	17y	43.514	35.548	-7.966
Adult +RT	21y	45.017	34.108	-10.909
Adult +RT	25y	45.128	35.476	-9.652
Adult +RT	31y	42.238	32.956	-9.282
Adult +RT	36y	41.610	32.429	-9.181
Adult +RT	40y	40.757	34.752	-6.005
Adult +RT	45y	47.069	34.282	-12.787
Middle-Age +RT	49y	50.000	37.761	-12.239
Middle-Age +RT	53y	46.929	33.922	-13.007
Middle-Age +RT	57y	44.332	32.686	-11.646
Middle-Age +RT	60y	43.396	33.649	-9.747
Middle-Age +RT	63y	48.022	33.334	-14.688
Elderly +RT	66y	43.165	33.929	-9.236
Elderly +RT	69y	44.958	35.218	-9.740
Elderly +RT	72y	44.568	32.998	-11.570
Elderly +RT	74y	47.145	35.558	-11.587
Elderly +RT	76y	45.374	35.630	-9.744
Elderly +RT	79y	48.204	21.633	-26.571
Elderly +RT	81y	44.638	27.282	-17.356
Elderly +RT	84y	40.969	34.405	-6.564
Elderly +RT	86y	45.564	33.270	-12.294
Elderly +RT	89y	44.987	33.242	-11.745
Elderly +RT	91y	39.628	32.059	-7.569
Elderly +RT	92y	44.128	34.138	-9.990
Elderly +RT	102y	40.475	33.270	-7.205
DNA		50.000	50.000	0.000
NTC		50.000	50.000	0.000

MAD1L1-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	34.657	33.184	-1.473
Newborn +RT	1h	33.066	32.755	-0.311
Newborn +RT	2d	36.374	34.912	-1.462
Newborn +RT	8d	31.072	31.727	0.655
Newborn +RT	17d	31.434	30.577	-0.857
Newborn +RT	1m	32.413	33.488	1.075
Newborn +RT	1m	30.697	31.740	1.043
Newborn +RT	2m	33.176	33.156	-0.020
Newborn +RT	3m	32.052	33.406	1.354
Infant +RT	4m	33.452	33.402	-0.050
Infant +RT	5m	31.633	31.706	0.073
Infant +RT	7m	32.866	33.304	0.438
Infant +RT	9m	34.490	34.519	0.029
Toddler +RT	10m	31.316	33.520	2.204
Toddler +RT	14m	33.411	34.015	0.604
Toddler +RT	18m	32.456	33.363	0.907
Toddler +RT	21m	32.736	33.922	1.186
Toddler +RT	2y	32.733	33.921	1.188
Toddler +RT	3y	32.592	32.248	-0.344
Child +RT	4y	31.477	32.272	0.795
Child +RT	4y	32.245	34.458	2.213
Child +RT	5y	31.107	31.242	0.135
Child +RT	6y	34.500	34.311	-0.189
Child +RT	8y	34.194	34.962	0.768
Child +RT	9y	34.143	34.754	0.611
Child +RT	9y	33.163	34.156	0.993
Child +RT	12y	33.774	34.448	0.674
Child +RT	12y	32.587	33.080	0.493
Juvenile +RT	13y	33.190	31.698	-1.492
Juvenile +RT	14y	33.060	32.323	-0.737
Juvenile +RT	14y	32.396	32.899	0.503
Juvenile +RT	15y	32.993	33.420	0.427
Juvenile +RT	15y	32.454	31.812	-0.642
Juvenile +RT	16y	33.574	33.288	-0.286
Juvenile +RT	16y	32.666	32.948	0.282
Juvenile +RT	17y	33.963	33.686	-0.277
Juvenile +RT	18y	33.975	34.138	0.163
Adult +RT	19y	33.151	33.562	0.411
Adult +RT	21y	37.224	37.556	0.332
Adult +RT	23y	32.736	33.045	0.309
Adult +RT	24y	31.290	32.101	0.811
Adult +RT	25y	33.915	33.319	-0.596
Adult +RT	27y	33.964	34.138	0.174
Adult +RT	29y	33.441	32.180	-1.261
Adult +RT	29y	32.816	33.583	0.767
Adult +RT	31y	32.110	32.224	0.114
Adult +RT	35y	35.361	35.799	0.438
Adult +RT	35y	33.675	34.706	1.031

MAD1L1-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Adult +RT	36y	33.486	33.820	0.334
Adult +RT	36y	33.289	33.195	-0.094
Adult +RT	38y	32.470	32.599	0.129
Adult +RT	40y	32.029	33.245	1.216
Adult +RT	40y	32.114	33.744	1.630
Adult +RT	43y	34.106	34.803	0.697
Adult +RT	43y	33.830	34.155	0.325
Adult +RT	45y	33.346	32.395	-0.951
Elderly +RT	46y	35.252	37.904	2.652
Elderly -RT	47y	35.807	36.876	1.069
Middle-Age +RT	47y	33.711	34.854	1.143
Middle-Age +RT	49y	37.889	40.000	2.111
Middle-Age +RT	51y	35.641	39.000	3.359
Middle-Age +RT	53y	35.006	34.468	-0.538
Middle-Age +RT	53y	32.477	32.521	0.044
Middle-Age +RT	56y	34.712	35.610	0.898
Middle-Age +RT	56y	32.791	33.674	0.883
Middle-Age +RT	57y	33.684	34.341	0.657
Middle-Age +RT	58y	33.345	33.724	0.379
Middle-Age +RT	60y	32.946	33.010	0.064
Middle-Age +RT	61y	32.485	33.306	0.821
Middle-Age +RT	63y	33.063	31.862	-1.201
Middle-Age +RT	63y	32.158	31.917	-0.241
Elderly +RT	65y	33.872	33.400	-0.472
Elderly +RT	66y	32.819	33.518	0.699
Elderly +RT	68y	32.226	33.876	1.650
Elderly +RT	68y	33.649	34.080	0.431
Elderly +RT	69y	33.235	33.290	0.055
Elderly +RT	71y	33.436	32.865	-0.571
Elderly +RT	71y	34.692	33.247	-1.445
Elderly +RT	72y	34.417	34.376	-0.041
Elderly +RT	74y	33.026	33.457	0.431
Elderly +RT	76y	33.171	32.944	-0.227
Elderly +RT	76y	35.582	34.907	-0.675
Elderly +RT	79y	34.132	34.388	0.256
Elderly +RT	80y	32.481	33.025	0.544
Elderly +RT	81y	35.783	37.165	1.382
Elderly +RT	83y	35.448	34.711	-0.737
Elderly +RT	84y	31.489	32.333	0.844
Elderly +RT	84y	34.094	33.945	-0.149
Elderly +RT	86y	32.868	32.549	-0.319
Elderly +RT	89y	33.992	35.399	1.407
Elderly +RT	89y	33.943	34.345	0.402
Elderly +RT	91y	32.281	32.060	-0.221
Elderly +RT	92y	32.169	32.246	0.077
Elderly +RT	102y	32.414	31.917	-0.497
DNA		40.000	40.000	0.000
NTC		40.000	40.000	0.000

PDCD6-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	34.342	34.266	-0.076
Newborn +RT	1h	34.692	33.921	-0.771
Newborn +RT	2d	37.965	35.505	-2.460
Newborn +RT	8d	31.603	32.549	0.946
Newborn +RT	17d	33.914	35.286	1.372
Newborn +RT	1m	32.438	34.809	2.371
Newborn +RT	1m	32.918	35.856	2.938
Newborn +RT	2m	33.641	33.266	-0.375
Newborn +RT	3m	32.339	34.069	1.730
Infant +RT	4m	34.348	34.272	-0.076
Infant +RT	5m	32.652	32.741	0.089
Infant +RT	7m	32.871	34.650	1.779
Infant +RT	9m	35.446	35.856	0.410
Toddler +RT	10m	31.657	34.360	2.703
Toddler +RT	14m	35.100	34.404	-0.696
Toddler +RT	18m	32.384	33.219	0.835
Toddler +RT	21m	32.843	34.050	1.207
Toddler +RT	2y	34.142	33.457	-0.685
Toddler +RT	3y	32.026	33.264	1.238
Child +RT	4y	31.664	34.477	2.813
Child +RT	4y	33.540	37.211	3.671
Child +RT	5y	31.020	32.129	1.109
Child +RT	6y	33.472	35.373	1.901
Child +RT	8y	35.501	35.969	0.468
Child +RT	9y	35.913	38.910	2.997
Child +RT	9y	33.854	36.516	2.662
Child +RT	12y	34.478	34.962	0.484
Child +RT	12y	33.784	33.559	-0.225
Juvenile +RT	13y	33.601	33.435	-0.166
Juvenile +RT	14y	33.785	32.942	-0.843
Juvenile +RT	14y	32.669	30.514	-2.155
Juvenile +RT	15y	32.462	33.528	1.066
Juvenile +RT	15y	31.584	32.249	0.665
Juvenile +RT	16y	33.227	33.546	0.319
Juvenile +RT	16y	32.204	33.615	1.411
Juvenile +RT	17y	32.306	35.640	3.334
Juvenile +RT	18y	34.445	34.859	0.414
Adult +RT	19y	32.412	34.506	2.094
Adult +RT	21y	37.960	35.500	-2.460
Adult +RT	23y	33.679	33.807	0.128
Adult +RT	24y	30.666	32.623	1.957
Adult +RT	25y	34.548	33.863	-0.685
Adult +RT	27y	34.569	34.606	0.037
Adult +RT	29y	33.321	32.925	-0.396
Adult +RT	29y	34.837	34.101	-0.736
Adult +RT	31y	31.355	32.654	1.299
Adult +RT	35y	35.770	34.575	-1.195

PDCD6-S15 Titration

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Adult +RT	36y	35.388	34.184	-1.204
Adult +RT	36y	35.932	35.482	-0.450
Adult +RT	38y	33.189	32.762	-0.427
Adult +RT	40y	32.015	34.236	2.221
Adult +RT	40y	33.884	33.578	-0.306
Adult +RT	43y	34.821	34.720	-0.101
Adult +RT	43y	35.741	35.048	-0.693
Adult +RT	45y	32.372	33.387	1.015
Elderly +RT	46y	34.858	34.562	-0.296
Elderly -RT	47y	34.272	35.563	1.291
Middle-Age +RT	47y	35.365	33.391	-1.974
Middle-Age +RT	49y	35.881	37.456	1.575
Middle-Age +RT	51y	36.429	36.413	-0.016
Middle-Age +RT	53y	36.811	34.946	-1.865
Middle-Age +RT	53y	33.509	34.125	0.616
Middle-Age +RT	56y	35.961	34.567	-1.394
Middle-Age +RT	56y	33.675	34.466	0.791
Middle-Age +RT	57y	34.749	34.178	-0.571
Middle-Age +RT	58y	32.724	34.022	1.298
Middle-Age +RT	60y	33.282	33.259	-0.023
Middle-Age +RT	61y	32.480	33.699	1.219
Middle-Age +RT	63y	34.973	33.287	-1.686
Middle-Age +RT	63y	31.341	32.784	1.443
Elderly +RT	65y	35.832	34.261	-1.571
Elderly +RT	66y	33.469	34.411	0.942
Elderly +RT	68y	33.699	33.802	0.103
Elderly +RT	68y	33.303	35.199	1.896
Elderly +RT	69y	33.738	33.833	0.095
Elderly +RT	71y	34.927	34.045	-0.882
Elderly +RT	71y	33.130	33.765	0.635
Elderly +RT	72y	34.257	33.965	-0.292
Elderly +RT	74y	35.264	35.555	0.291
Elderly +RT	76y	33.765	33.498	-0.267
Elderly +RT	76y	35.292	34.823	-0.469
Elderly +RT	79y	35.382	33.889	-1.493
Elderly +RT	80y	34.087	33.718	-0.369
Elderly +RT	81y	35.635	35.539	-0.096
Elderly +RT	83y	36.555	35.441	-1.114
Elderly +RT	84y	33.128	33.305	0.177
Elderly +RT	84y	33.061	33.669	0.608
Elderly +RT	86y	34.494	33.554	-0.940
Elderly +RT	89y	34.224	33.479	-0.745
Elderly +RT	89y	34.818	34.106	-0.712
Elderly +RT	91y	31.701	32.947	1.246
Elderly +RT	92y	34.167	32.953	-1.214
Elderly +RT	102y	32.567	33.526	0.959
DNA		50.000	50.000	0.000

Table 7: COL1A2 Real-Time PCR Singleplex Candidate Results.

COL1A2-Initial Amplification

Sample #	Sex	Age	Ct value
Newborn +RT	M	1h	38.791
Newborn -RT			Undet
Newborn +RT	F	17d	36.887
Newborn -RT			Undet
Newborn +RT	F	1m	41.468
Newborn -RT			Undet
Newborn +RT	F	3m	37.954
Newborn -RT			Undet
Infant +RT	F	5m	50.000
Infant -RT			Undet
Infant +RT	M	8m	45.571
Infant -RT			Undet
Toddler +RT	F	14m	50.000
Toddler -RT			Undet
Toddler +RT	F	19m	40.946
Toddler -RT			Undet
Toddler +RT	M	2.8y	50.000
Toddler -RT			Undet
Child +RT	M	4y	38.423
Child -RT			Undet
Child +RT	F	4y	46.277
Child -RT			Undet

COL1A2-Initial Amplification

Sample #	Sex	Age	Ct value
Child +RT	F	6y	50.000
Child -RT			Undet
Child +RT	F	12y	41.147
Child -RT			Undet
Juvenile +RT	M	18y	50.000
Juvenile -RT			Undet
Adult +RT	M	27y	50.000
Adult -RT			Undet
Adult +RT	M	40y	50.000
Adult -RT			Undet
Middle-Age +RT	M	51y	50.000
Middle-Age -RT			Undet
Middle-Age +RT	M	61y	50.000
Middle-Age -RT			Undet
Elderly +RT	M	76y	50.000
Elderly -RT			Undet
Elderly +RT	M	86y	50.000
Elderly -RT			Undet
DNA			Undet
DNA			Undet
NTC			Undet
NTC			Undet

Table 8: COL1A2 Real-Time PCR Duplex Delta Ct Results.

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value	Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	45.256	50.000	4.744	Adult +RT	36y	50.000	48.825	-1.175
Newborn +RT	1h	40.197	50.000	9.803	Adult +RT	36y	50.000	43.970	-6.030
Newborn +RT	2d	44.296	50.000	5.704	Adult +RT	38y	42.611	39.645	-2.966
Newborn +RT	8d	36.656	50.000	13.344	Adult +RT	40y	50.000	41.516	-8.484
Newborn +RT	17d	41.478	48.714	7.236	Adult +RT	40y	50.000	40.792	-9.208
Newborn +RT	1m	39.251	47.951	8.700	Adult +RT	43y	50.000	41.325	-8.675
Newborn +RT	1m	50.000	50.000	0.000	Adult +RT	43y	50.000	42.427	-7.573
Newborn +RT	2m	40.341	46.056	5.715	Adult +RT	45y	50.000	38.497	-11.503
Newborn +RT	3m	38.706	47.180	8.474	Elderly +RT	46y	50.000	40.595	-9.405
Infant +RT	4m	43.222	43.090	-0.132	Elderly -RT	47y	41.897	43.741	1.844
Infant +RT	5m	43.497	41.358	-2.139	Middle-Age +RT	47y	50.000	38.891	-11.109
Infant +RT	7m	43.332	42.439	-0.893	Middle-Age +RT	49y	50.000	44.039	-5.961
Infant +RT	9m	50.000	50.000	0.000	Middle-Age +RT	51y	50.000	41.160	-8.840
Toddler +RT	10m	41.271	48.489	7.218	Middle-Age +RT	53y	50.000	40.988	-9.012
Toddler +RT	14m	50.000	45.904	-4.096	Middle-Age +RT	53y	50.000	50.000	0.000
Toddler +RT	18m	50.000	43.718	-6.282	Middle-Age +RT	56y	50.000	41.875	-8.125
Toddler +RT	21m	50.000	43.215	-6.785	Middle-Age +RT	56y	50.000	40.611	-9.389
Toddler +RT	2y	50.000	42.368	-7.632	Middle-Age +RT	57y	50.000	41.501	-8.499
Toddler +RT	3y	50.000	41.687	-8.313	Middle-Age +RT	58y	50.000	40.335	-9.665
Child +RT	4y	41.864	39.589	-2.275	Middle-Age +RT	60y	50.000	42.757	-7.243
Child +RT	4y	40.689	42.658	1.969	Middle-Age +RT	61y	43.118	50.000	6.882
Child +RT	5y	39.318	39.088	-0.230	Middle-Age +RT	63y	50.000	38.031	-11.969
Child +RT	6y	50.000	40.970	-9.030	Middle-Age +RT	63y	42.383	36.639	-5.744
Child +RT	8y	44.289	50.000	5.711	Elderly +RT	65y	50.000	42.035	-7.965
Child +RT	9y	50.000	50.000	0.000	Elderly +RT	66y	50.000	47.742	-2.258
Child +RT	9y	50.000	46.608	-3.392	Elderly +RT	68y	42.262	37.598	-4.664
Child +RT	12y	50.000	46.480	-3.520	Elderly +RT	68y	50.000	44.386	-5.614
Child +RT	12y	50.000	42.175	-7.825	Elderly +RT	69y	50.000	41.053	-8.947
Juvenile +RT	13y	43.040	41.343	-1.697	Elderly +RT	71y	46.120	50.000	3.880
Juvenile +RT	14y	50.000	35.288	-14.712	Elderly +RT	71y	50.000	41.181	-8.819
Juvenile +RT	14y	45.600	40.692	-4.908	Elderly +RT	72y	50.000	50.000	0.000
Juvenile +RT	15y	41.540	39.939	-1.601	Elderly +RT	74y	50.000	41.608	-8.392
Juvenile +RT	15y	50.000	38.983	-11.017	Elderly +RT	76y	43.599	40.798	-2.801
Juvenile +RT	16y	50.000	39.755	-10.245	Elderly +RT	76y	50.000	40.631	-9.369
Juvenile +RT	16y	50.000	39.832	-10.168	Elderly +RT	79y	50.000	50.000	0.000
Juvenile +RT	17y	50.000	40.995	-9.005	Elderly +RT	80y	50.000	38.305	-11.695
Juvenile +RT	18y	42.170	46.590	4.420	Elderly +RT	81y	50.000	42.766	-7.234
Adult +RT	19y	50.000	43.591	-6.409	Elderly +RT	83y	50.000	45.156	-4.844
Adult +RT	21y	50.000	43.065	-6.935	Elderly +RT	84y	50.000	38.506	-11.494
Adult +RT	23y	50.000	50.000	0.000	Elderly +RT	84y	50.000	50.000	0.000
Adult +RT	24y	50.000	39.744	-10.256	Elderly +RT	86y	46.010	50.000	3.990
Adult +RT	25y	50.000	40.392	-9.608	Elderly +RT	89y	50.000	43.349	-6.651
Adult +RT	27y	43.390	42.501	-0.889	Elderly +RT	89y	50.000	42.636	-7.364
Adult +RT	29y	50.000	38.550	-11.450	Elderly +RT	91y	50.000	39.317	-10.683
Adult +RT	29y	50.000	40.104	-9.896	Elderly +RT	92y	50.000	40.486	-9.514
Adult +RT	31y	50.000	39.025	-10.975	Elderly +RT	102y	50.000	50.000	0.000
Adult +RT	35y	50.000	41.378	-8.622	DNA		50.000	50.000	0.000
Adult +RT	35y	50.000	41.799	-8.201	NTC		50.000	50.000	0.000

Table 9: COL1A2 Triplicate qPCR Results.

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Newborn +RT	1h	43.886	1.290	50.000	0.000	6.114	1.290
Newborn +RT	1h	41.552	0.193	50.000	0.000	8.448	0.193
Newborn +RT	1h	42.532	0.790	46.961	1.715	4.429	1.160
Newborn +RT	1h	41.648	1.889	50.000	0.000	8.352	1.889
Newborn +RT	1h	40.896	0.621	50.000	0.000	9.104	0.621
Newborn +RT	1h	43.963	0.384	50.000	0.000	6.037	0.384
Newborn +RT	1h	40.668	0.493	50.000	0.000	9.332	0.493
Newborn +RT	1d	38.139	0.311	50.000	0.000	11.861	0.311
Newborn +RT	2d	43.854	1.189	49.848	0.263	5.994	1.078
Newborn +RT	8d	37.388	0.244	50.000	0.000	12.612	0.244
Newborn +RT	13d	40.033	1.135	50.000	0.000	9.967	1.135
Newborn +RT	17d	40.004	1.114	43.424	2.341	3.420	1.439
Infant +RT	1m	40.222	0.630	50.000	0.000	9.778	0.630
Infant +RT	1m	44.994	4.372	43.056	1.175	-1.938	3.385
Infant +RT	2m	39.650	0.616	48.100	1.286	8.449	1.892
Infant +RT	3m	38.037	0.280	41.981	0.694	3.945	0.431
Infant +RT	3m	40.706	1.246	50.000	0.000	9.294	1.246
Infant +RT	4m	47.282	4.708	44.045	1.226	-3.237	5.204
Infant +RT	4m	39.991	1.852	50.000	0.000	10.009	1.852
Infant +RT	5m	42.121	1.174	45.112	1.271	2.991	0.378
Toddler +RT	6m	50.000	0.000	45.677	1.030	-4.323	1.030
Toddler +RT	7m	50.000	0.000	41.693	1.283	-8.307	1.283
Toddler +RT	7m	50.000	0.000	41.922	1.318	-8.078	1.318
Toddler +RT	7m	42.385	0.670	50.000	0.000	7.615	0.670
Toddler +RT	8m	44.901	4.426	46.547	2.214	1.646	2.356
Toddler +RT	8m	50.000	0.000	47.171	0.518	-2.829	0.518
Toddler +RT	8m	45.496	4.061	42.334	1.998	-3.162	3.044
Toddler +RT	9m	40.543	1.171	48.134	1.807	7.592	2.970
Toddler +RT	9m	50.000	0.000	43.834	0.681	-6.166	0.681
Toddler +RT	10m	50.000	0.000	46.348	2.491	-3.652	2.491
Toddler +RT	10m	39.394	0.536	44.314	0.992	4.920	0.459
Toddler +RT	14m	50.000	0.000	43.159	0.617	-6.841	0.617
Toddler +RT	15m	50.000	0.000	43.057	1.172	-6.943	1.172
Toddler +RT	18m	50.000	0.000	43.093	2.356	-6.907	2.356
Toddler +RT	19m	45.367	4.036	48.678	1.170	3.311	4.813
Toddler +RT	21m	50.000	0.000	43.164	0.770	-6.836	0.770
Toddler +RT	2y	50.000	0.000	42.657	0.418	-7.343	0.418
Toddler +RT	2.8y	50.000	0.000	43.486	2.171	-6.514	2.171
Toddler +RT	3y	41.396	0.547	41.065	0.583	-0.331	1.094
Toddler +RT	3y	50.000	0.000	42.255	0.690	-7.745	0.690

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Child +RT	4y	41.201	1.019	43.054	1.020	1.853	0.395
Child +RT	4y	42.100	0.724	43.757	0.360	1.658	0.618
Child +RT	4y	40.839	0.594	43.962	1.713	3.123	2.140
Child +RT	5y	39.351	0.687	39.363	2.269	0.011	1.961
Child +RT	6y	50.000	0.000	41.293	3.938	-8.707	3.938
Child +RT	8y	44.933	4.405	47.211	2.459	2.278	6.719
Child +RT	9y	50.000	0.000	43.840	0.935	-6.160	0.935
Child +RT	12y	50.000	0.000	45.390	1.553	-4.610	1.553
Child +RT	12y	50.000	0.000	40.897	1.515	-9.103	1.515
Child +RT	12y	50.000	0.000	43.476	0.786	-6.524	0.786
Child +RT	12y	46.861	5.437	41.560	2.573	-5.301	5.369
Juvenile +RT	13y	50.000	0.000	41.881	2.569	-8.119	2.569
Juvenile +RT	13y	50.000	0.000	42.489	0.726	-7.511	0.726
Juvenile +RT	14y	50.000	0.000	42.667	4.225	-7.333	4.225
Juvenile +RT	14y	50.000	0.000	38.952	2.576	-11.048	2.576
Juvenile +RT	14y	50.000	0.000	43.646	1.057	-6.354	1.057
Juvenile +RT	15y	50.000	0.000	42.970	2.163	-7.030	2.163
Juvenile +RT	15y	41.335	1.673	44.333	1.620	2.998	3.282
Juvenile +RT	15y	50.000	0.000	42.818	1.550	-7.182	1.550
Juvenile +RT	16y	45.302	4.078	45.432	1.115	0.130	3.578
Juvenile +RT	17y	50.000	0.000	44.379	2.201	-5.621	2.201
Juvenile +RT	17y	50.000	0.000	44.544	2.009	-5.456	2.009
Juvenile +RT	17y	50.000	0.000	45.018	1.796	-4.982	1.796
Juvenile +RT	18y	50.000	0.000	43.583	1.020	-6.417	1.020
Juvenile +RT	18y	45.147	4.248	43.758	2.022	-1.389	5.280
Adult +RT	19y	48.024	3.423	47.186	2.761	-0.838	6.002
Adult +RT	24y	50.000	0.000	45.658	1.423	-4.342	1.423
Adult +RT	26y	50.000	0.000	44.300	1.074	-5.700	1.074
Adult +RT	29y	50.000	0.000	43.917	2.233	-6.083	2.233
Adult +RT	29y	50.000	0.000	45.175	0.642	-4.825	0.642
Adult +RT	35y	50.000	0.000	44.768	0.613	-5.232	0.613
Adult +RT	35y	50.000	0.000	46.108	1.467	-3.892	1.467
Adult +RT	36y	50.000	0.000	44.930	1.783	-5.070	1.783
Adult +RT	38y	50.000	0.000	41.480	0.415	-8.520	0.415
Adult +RT	38y	50.000	0.000	42.031	1.055	-7.969	1.055
Adult +RT	38y	47.561	4.224	45.297	4.075	-2.264	8.299
Adult +RT	40y	48.983	1.761	37.840	3.453	-11.144	2.237
Adult +RT	40y	50.000	0.000	42.796	0.507	-7.204	0.507
Adult +RT	43y	50.000	0.000	44.101	0.756	-5.899	0.756
Adult +RT	45y	44.696	4.642	42.578	1.399	-2.119	3.873
Adult +RT	45y	50.000	0.000	42.193	0.528	-7.807	0.528
Adult +RT	45y	41.079	0.315	43.518	1.719	2.438	2.023

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Middle-Age +RT	47y	47.656	4.059	42.680	2.010	-4.976	5.906
Middle-Age +RT	51y	50.000	0.000	43.148	1.283	-6.852	1.283
Middle-Age +RT	53y	50.000	0.000	46.940	0.592	-3.060	0.592
Middle-Age +RT	53y	50.000	0.000	43.976	1.983	-6.024	1.983
Middle-Age +RT	56y	50.000	0.000	42.970	1.093	-7.030	1.093
Middle-Age +RT	57y	50.000	0.000	46.294	1.531	-3.706	1.531
Middle-Age +RT	58y	50.000	0.000	42.681	1.219	-7.319	1.219
Middle-Age +RT	59y	50.000	0.000	42.914	0.527	-7.086	0.527
Middle-Age +RT	60y	50.000	0.000	43.518	0.730	-6.482	0.730
Middle-Age +RT	61y	48.101	3.289	46.222	0.627	-1.880	3.916
Middle-Age +RT	61y	41.985	0.636	49.018	1.701	7.033	1.400
Middle-Age +RT	63y	50.000	0.000	44.614	0.734	-5.386	0.734
Middle-Age +RT	63y	42.738	1.602	42.804	0.414	0.066	1.225
Elderly +RT	65y	50.000	0.000	44.799	4.573	-5.201	4.573
Elderly +RT	68y	42.180	0.244	50.000	0.000	7.820	0.244
Elderly +RT	71y	47.878	3.675	42.159	0.920	-5.719	4.532
Elderly +RT	71y	50.000	0.000	44.437	2.132	-5.563	2.132
Elderly +RT	76y	50.000	0.000	45.017	1.156	-4.983	1.156
Elderly +RT	76y	50.000	0.000	46.260	1.787	-3.740	1.787
Elderly +RT	80y	50.000	0.000	42.367	1.702	-7.633	1.702
Elderly +RT	81y	50.000	0.000	42.744	1.182	-7.256	1.182
Elderly +RT	84y	50.000	0.000	42.006	1.987	-7.994	1.987
Elderly +RT	86y	47.308	4.663	44.190	0.133	-3.117	4.625
Elderly +RT	89y	50.000	0.000	43.455	2.898	-6.545	2.898
Elderly +RT	89y	47.564	4.219	47.109	3.505	-0.455	7.156
Elderly +RT	92y	50.000	0.000	41.896	1.166	-8.104	1.166
Elderly +RT	102y	50.000	0.000	42.832	1.240	-7.168	1.240

Table 10: HBE1 Real-Time PCR Singleplex Candidate Results.

HBE1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	F	1h	0.003	28.615
Newborn -RT				Undet
Newborn +RT	M	1h	0.003	28.041
Newborn -RT				Undet
Newborn +RT	F	17d	0.047	32.659
Newborn -RT				Undet
Newborn +RT	M	2m	0.167	33.464
Newborn -RT				Undet
Toddler +RT	M	10m	0.833	35.566
Toddler -RT				Undet
Toddler +RT	F	3y	3.000	35.926
Toddler -RT				Undet
Child +RT	M	8y	8.000	39.075
Child -RT				Undet

HBE1-Initial Amplification

Sample #	Sex	Age	Age (Yrs)	Ct value
Juvenile +RT	M	16y	16.000	34.536
Juvenile -RT				Undet
Adult +RT	F	35y	35.000	38.329
Adult -RT				Undet
Middle-Age +RT	M	56y	56.000	32.677
Middle-Age -RT				Undet
Elderly +RT	F	69y	69.000	33.112
Elderly -RT				Undet
Elderly +RT	M	80y	80.000	33.140
Elderly -RT				Undet
DNA				Undet
DNA				Undet
NTC				Undet
NTC				Undet

Table 11: HBE1 Real-Time PCR Duplex Delta Ct Results.

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value	Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	30.334	35.600	5.266	Adult +RT	36y	33.663	34.571	0.908
Newborn +RT	1h	29.454	36.348	6.894	Adult +RT	36y	34.011	36.938	2.927
Newborn +RT	2d	31.497	36.344	4.847	Adult +RT	38y	33.939	33.635	-0.304
Newborn +RT	8d	28.742	33.151	4.409	Adult +RT	40y	36.087	33.531	-2.556
Newborn +RT	17d	36.419	34.528	-1.891	Adult +RT	40y	34.268	33.344	-0.924
Newborn +RT	1m	31.793	34.283	2.490	Adult +RT	43y	35.758	34.431	-1.327
Newborn +RT	1m	26.481	35.650	9.169	Adult +RT	43y	36.427	34.102	-2.325
Newborn +RT	2m	34.816	34.115	-0.701	Adult +RT	45y	31.847	31.266	-0.581
Newborn +RT	3m	31.477	36.650	5.173	Elderly +RT	46y	37.779	34.190	-3.589
Infant +RT	4m	36.739	33.756	-2.983	Elderly -RT	47y	39.020	35.466	-3.554
Infant +RT	5m	34.472	32.444	-2.028	Middle-Age +RT	47y	33.377	33.568	0.191
Infant +RT	7m	37.068	33.692	-3.376	Middle-Age +RT	49y	32.288	41.326	9.038
Infant +RT	9m	35.862	35.536	-0.326	Middle-Age +RT	51y	34.377	36.995	2.618
Toddler +RT	10m	39.769	33.174	-6.595	Middle-Age +RT	53y	33.496	36.227	2.731
Toddler +RT	14m	36.301	33.809	-2.492	Middle-Age +RT	53y	32.336	33.344	1.008
Toddler +RT	18m	40.080	33.092	-6.988	Middle-Age +RT	56y	32.820	34.858	2.038
Toddler +RT	21m	37.282	33.368	-3.914	Middle-Age +RT	56y	34.211	34.193	-0.018
Toddler +RT	2y	33.388	33.307	-0.081	Middle-Age +RT	57y	36.067	33.589	-2.478
Toddler +RT	3y	39.098	32.606	-6.492	Middle-Age +RT	58y	33.307	33.603	0.296
Child +RT	4y	40.236	32.509	-7.727	Middle-Age +RT	60y	36.616	32.399	-4.217
Child +RT	4y	32.761	30.136	-2.625	Middle-Age +RT	61y	33.694	33.490	-0.204
Child +RT	5y	34.820	31.375	-3.445	Middle-Age +RT	63y	35.233	34.140	-1.093
Child +RT	6y	38.535	34.825	-3.710	Middle-Age +RT	63y	32.970	33.969	0.999
Child +RT	8y	39.831	35.021	-4.810	Elderly +RT	65y	33.069	34.779	1.710
Child +RT	9y	38.202	34.347	-3.855	Elderly +RT	66y	34.380	33.692	-0.688
Child +RT	9y	39.585	35.636	-3.949	Elderly +RT	68y	34.162	36.358	2.196
Child +RT	12y	38.094	34.787	-3.307	Elderly +RT	68y	37.887	34.393	-3.494
Child +RT	12y	34.692	33.182	-1.510	Elderly +RT	69y	35.421	33.888	-1.533
Juvenile +RT	13y	33.955	33.331	-0.624	Elderly +RT	71y	34.532	33.328	-1.204
Juvenile +RT	14y	38.146	32.170	-5.976	Elderly +RT	71y	34.341	34.017	-0.324
Juvenile +RT	14y	38.379	32.333	-6.046	Elderly +RT	72y	36.528	34.682	-1.846
Juvenile +RT	15y	36.141	32.941	-3.200	Elderly +RT	74y	32.297	34.526	2.229
Juvenile +RT	15y	36.014	32.498	-3.516	Elderly +RT	76y	32.672	33.363	0.691
Juvenile +RT	16y	34.429	33.673	-0.756	Elderly +RT	76y	33.382	34.562	1.180
Juvenile +RT	16y	35.811	33.060	-2.751	Elderly +RT	79y	35.049	33.391	-1.658
Juvenile +RT	17y	37.634	34.156	-3.478	Elderly +RT	80y	33.648	33.877	0.229
Juvenile +RT	18y	36.059	34.722	-1.337	Elderly +RT	81y	34.027	35.334	1.307
Adult +RT	19y	36.039	33.675	-2.364	Elderly +RT	83y	35.877	34.875	-1.002
Adult +RT	21y	33.974	36.982	3.008	Elderly +RT	84y	38.792	33.043	-5.749
Adult +RT	23y	36.217	33.207	-3.010	Elderly +RT	84y	36.862	33.858	-3.004
Adult +RT	24y	35.201	32.410	-2.791	Elderly +RT	86y	35.139	33.535	-1.604
Adult +RT	25y	34.418	33.117	-1.301	Elderly +RT	89y	36.299	32.876	-3.423
Adult +RT	27y	32.107	33.770	1.663	Elderly +RT	89y	34.405	34.331	-0.074
Adult +RT	29y	33.602	31.966	-1.636	Elderly +RT	91y	37.291	32.856	-4.435
Adult +RT	29y	33.783	34.439	0.656	Elderly +RT	92y	31.672	33.053	1.381
Adult +RT	31y	37.976	32.440	-5.536	Elderly +RT	102y	34.552	33.734	-0.818
Adult +RT	35y	38.910	34.427	-4.483	DNA		50.000	50.000	0.000
Adult +RT	35y	33.125	35.161	2.036	NTC		50.000	50.000	0.000

Table 12: HBE1 Triplicate qPCR Results.

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Newborn +RT	1h	34.639	0.461	35.726	0.698	1.087	0.298
Newborn +RT	1h	30.023	0.337	33.593	0.221	3.571	0.123
Newborn +RT	1h	30.427	0.179	34.385	0.237	3.959	0.133
Newborn +RT	1h	28.889	0.177	32.478	0.284	3.589	0.111
Newborn +RT	1h	28.890	0.159	33.646	0.421	4.756	0.378
Newborn +RT	1h	29.318	0.114	35.112	0.233	5.793	0.303
Newborn +RT	1h	28.733	0.277	37.526	0.888	8.793	0.672
Newborn +RT	1d	31.367	0.148	32.956	0.141	1.590	0.240
Newborn +RT	2d	31.126	0.097	35.821	1.042	4.694	0.968
Newborn +RT	8d	28.776	0.181	32.911	0.373	4.136	0.193
Newborn +RT	13d	28.713	0.380	32.743	0.252	4.030	0.626
Newborn +RT	17d	33.336	0.355	32.688	0.255	-0.647	0.116
Newborn +RT	1m	32.845	0.363	33.944	0.574	1.099	0.264
Newborn +RT	1m	27.493	0.112	32.049	0.028	4.556	0.084
Newborn +RT	2m	33.575	0.145	32.343	0.118	-1.233	0.100
Newborn +RT	3m	30.002	0.110	32.469	0.082	2.467	0.096
Newborn +RT	3m	34.602	0.323	34.862	0.641	0.260	0.461
Infant +RT	4m	36.031	0.307	32.667	0.116	-3.364	0.422
Infant +RT	4m	34.593	0.456	31.943	0.084	-2.651	0.481
Infant +RT	5m	36.198	1.215	32.806	0.325	-3.392	0.955
Infant +RT	5m	37.554	0.949	33.829	0.774	-3.725	0.357
Infant +RT	6m	35.244	0.459	32.742	0.283	-2.501	0.405
Infant +RT	7m	36.819	0.745	31.405	0.246	-5.414	0.628
Infant +RT	7m	33.533	0.733	32.645	0.520	-0.887	1.094
Infant +RT	7m	34.485	0.080	33.470	0.128	-1.016	0.132
Infant +RT	8m	36.568	1.072	32.066	0.334	-4.502	1.233
Infant +RT	8m	34.552	0.487	35.270	0.248	0.718	0.378
Infant +RT	8m	35.368	0.451	31.318	0.129	-4.050	0.353
Infant +RT	9m	38.327	0.617	32.295	0.145	-6.031	0.593
Infant +RT	9m	36.243	0.241	33.606	0.382	-2.637	0.596
Toddler +RT	10m	37.774	0.376	32.868	0.453	-4.905	0.300
Toddler +RT	10m	39.078	0.351	32.596	0.225	-6.482	0.573
Toddler +RT	14m	36.184	0.330	33.664	0.329	-2.520	0.328
Toddler +RT	14m	36.721	0.377	34.908	0.252	-1.814	0.324
Toddler +RT	15m	35.537	0.540	33.814	0.521	-1.723	0.327
Toddler +RT	18m	37.507	0.550	32.686	0.288	-4.820	0.600
Toddler +RT	19m	35.122	0.347	32.914	0.349	-2.207	0.274
Toddler +RT	21m	36.986	0.522	33.089	0.322	-3.897	0.537
Toddler +RT	2y	35.672	0.668	33.690	0.460	-1.982	0.208
Toddler +RT	2.8y	34.992	0.119	33.458	0.298	-1.534	0.324
Toddler +RT	3y	40.691	0.881	32.838	0.170	-7.853	0.818
Toddler +RT	3y	36.095	0.234	31.764	0.249	-4.330	0.251

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Child +RT	4y	33.239	0.453	32.537	0.156	-0.702	0.317
Child +RT	4y	39.946	0.764	31.998	0.508	-7.948	0.513
Child +RT	4y	35.054	0.644	33.583	0.318	-1.471	0.357
Child +RT	5y	35.224	0.115	30.800	0.286	-4.424	0.342
Child +RT	6y	37.249	0.683	32.361	0.522	-4.888	0.279
Child +RT	6y	38.796	0.880	33.669	0.237	-5.127	0.670
Child +RT	8y	38.383	0.337	34.840	0.289	-3.544	0.614
Child +RT	9y	37.478	0.683	33.591	0.362	-3.887	0.787
Child +RT	9y	38.319	0.255	34.516	0.500	-3.803	0.704
Child +RT	12y	38.077	1.369	33.992	0.358	-4.085	1.353
Child +RT	12y	35.012	0.259	32.411	0.286	-2.601	0.230
Child +RT	12y	35.721	0.126	32.905	0.307	-2.816	0.264
Child +RT	12y	38.174	0.839	33.580	0.486	-4.594	0.353
Juvenile +RT	13y	36.579	0.280	34.183	0.430	-2.396	0.513
Juvenile +RT	13y	38.910	0.682	32.542	0.093	-6.368	0.670
Juvenile +RT	14y	41.041	0.400	33.374	0.449	-7.667	0.848
Juvenile +RT	14y	39.090	0.911	32.994	0.308	-6.096	0.926
Juvenile +RT	14y	42.575	1.481	33.743	0.320	-8.832	1.362
Juvenile +RT	15y	34.271	0.271	30.532	0.048	-3.739	0.283
Juvenile +RT	15y	34.957	0.184	32.950	0.183	-2.007	0.151
Juvenile +RT	15y	36.975	0.799	32.968	0.755	-4.007	0.964
Juvenile +RT	16y	38.098	0.431	32.904	0.617	-5.194	0.416
Juvenile +RT	16y	36.090	0.359	33.343	0.150	-2.747	0.337
Juvenile +RT	17y	33.497	0.619	33.619	0.416	0.122	0.213
Juvenile +RT	17y	32.082	0.586	35.314	0.521	3.232	0.135
Juvenile +RT	17y	33.156	0.229	33.205	0.280	0.049	0.171
Juvenile +RT	18y	33.952	0.236	31.615	0.384	-2.337	0.192
Juvenile +RT	18y	34.323	0.476	32.079	0.796	-2.244	0.361

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Adult +RT	19y	37.761	0.408	34.610	0.399	-3.151	0.268
Adult +RT	19y	37.348	1.080	33.452	0.309	-3.896	0.947
Adult +RT	21y	32.957	0.570	33.217	1.370	0.260	0.902
Adult +RT	21y	33.743	0.286	32.924	0.639	-0.819	0.366
Adult +RT	22y	36.813	0.377	34.482	0.247	-2.331	0.131
Adult +RT	22y	36.532	0.144	33.301	0.093	-3.231	0.221
Adult +RT	23y	37.093	0.734	32.734	0.288	-4.359	0.448
Adult +RT	24y	36.691	0.341	32.084	0.226	-4.607	0.424
Adult +RT	25y	37.374	0.951	36.054	1.148	-1.320	0.224
Adult +RT	26y	34.100	0.356	32.009	0.288	-2.091	0.635
Adult +RT	26y	40.495	1.415	33.920	0.273	-6.576	1.295
Adult +RT	27y	32.948	0.193	33.828	0.326	0.881	0.145
Adult +RT	29y	36.890	1.238	33.546	1.173	-3.344	0.430
Adult +RT	29y	36.447	0.257	34.180	0.221	-2.267	0.140
Adult +RT	35y	37.427	0.645	34.180	0.022	-3.248	0.659
Adult +RT	35y	33.871	0.573	34.156	0.276	0.285	0.346
Adult +RT	36y	37.773	0.636	33.359	0.547	-4.414	0.719
Adult +RT	36y	32.424	0.315	33.371	0.285	0.947	0.097
Adult +RT	38y	32.911	0.102	31.941	0.365	-0.970	0.293
Adult +RT	38y	34.937	0.350	32.880	0.376	-2.057	0.207
Adult +RT	38y	36.152	0.350	33.664	0.173	-2.487	0.182
Adult +RT	40y	36.754	0.228	33.308	0.564	-3.446	0.372
Adult +RT	40y	34.180	0.584	32.989	0.340	-1.191	0.287
Adult +RT	43y	35.334	0.776	34.551	0.341	-0.783	0.444
Adult +RT	43y	34.337	0.682	34.247	0.283	-0.091	0.645
Adult +RT	45y	33.000	0.390	33.300	0.261	0.300	0.193
Adult +RT	45y	33.019	0.377	32.757	0.389	-0.262	0.763
Adult +RT	45y	37.822	1.606	34.479	1.697	-3.343	0.688

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Middle-Age +RT	46y	36.400	0.623	33.405	0.202	-2.995	0.769
Middle-Age +RT	47y	34.379	0.427	33.193	0.600	-1.186	0.354
Middle-Age +RT	47y	39.753	0.844	34.446	1.049	-5.307	0.205
Middle-Age +RT	51y	33.966	0.203	33.329	0.224	-0.638	0.124
Middle-Age +RT	53y	34.616	0.868	33.152	0.608	-1.464	0.352
Middle-Age +RT	53y	34.537	0.205	34.095	0.641	-0.442	0.762
Middle-Age +RT	56y	33.200	0.737	34.682	0.846	1.483	0.879
Middle-Age +RT	56y	34.232	0.523	33.719	0.679	-0.513	0.657
Middle-Age +RT	57y	36.640	0.572	34.428	0.384	-2.212	0.224
Middle-Age +RT	58y	35.420	0.533	35.219	0.943	-0.201	0.576
Middle-Age +RT	59y	34.343	0.084	33.297	0.285	-1.046	0.201
Middle-Age +RT	60y	36.811	0.356	32.951	1.291	-3.860	1.308
Middle-Age +RT	61y	29.949	0.182	32.494	0.518	2.545	0.381
Middle-Age +RT	61y	35.798	0.576	34.858	0.330	-0.940	0.621
Middle-Age +RT	63y	31.241	0.173	31.172	0.306	-0.069	0.140
Middle-Age +RT	63y	32.556	0.173	32.255	0.271	-0.301	0.165
Middle-Age +RT	65y	31.965	0.177	33.168	0.131	1.202	0.060
Elderly +RT	66y	37.121	0.312	35.042	0.140	-2.079	0.231
Elderly +RT	66y	38.308	0.353	33.882	0.168	-4.425	0.468
Elderly +RT	68y	36.408	0.562	34.371	0.391	-2.037	0.203
Elderly +RT	68y	36.392	0.987	33.782	0.395	-2.610	1.261
Elderly +RT	69y	35.221	0.280	33.547	0.473	-1.674	0.248
Elderly +RT	71y	34.893	0.579	35.971	0.431	1.078	0.168
Elderly +RT	71y	34.382	0.194	33.246	0.414	-1.136	0.249
Elderly +RT	71y	35.324	0.213	33.338	0.286	-1.985	0.459
Elderly +RT	72y	36.617	0.190	34.322	0.366	-2.295	0.177
Elderly +RT	74y	32.468	0.160	33.839	0.424	1.371	0.539
Elderly +RT	76y	34.318	0.397	33.890	0.431	-0.428	0.148
Elderly +RT	76y	35.705	0.610	34.568	0.323	-1.137	0.357
Elderly +RT	79y	34.739	0.143	34.091	1.088	-0.648	1.187
Elderly +RT	80y	33.629	0.216	32.656	0.064	-0.974	0.258
Elderly +RT	81y	31.964	0.189	32.923	0.216	0.959	0.220
Elderly +RT	83y	34.704	0.540	33.695	0.020	-1.008	0.555
Elderly +RT	84y	36.085	0.111	33.056	0.478	-3.029	0.368
Elderly +RT	84y	37.379	0.600	33.194	0.307	-4.185	0.326
Elderly +RT	86y	36.039	0.762	33.934	1.445	-2.105	0.689
Elderly +RT	89y	35.420	1.554	32.444	0.807	-2.976	0.834
Elderly +RT	89y	36.970	0.761	33.363	0.180	-3.607	0.648
Elderly +RT	91y	37.940	0.380	35.794	0.614	-2.146	0.983
Elderly +RT	92y	30.063	0.143	31.410	0.270	1.347	0.160
Elderly +RT	102y	36.843	1.094	33.264	0.382	-3.579	0.823

Table 13: IGFBP3 Real-Time PCR Singleplex Candidate Results.

Sample #	Sex	Age	Age (Yrs)	Ct value	Sample #	Sex	Age	Age (Yrs)	Ct value
Newborn +RT	M	1h	0.003	40.000	Middle-Aged +RT	M	46y	46.000	36.918
Newborn -RT				Undet	Middle-Aged -RT				Undet
Newborn +RT	F	1m	0.083	40.000	Middle-Aged +RT	M	51y	51.000	37.031
Newborn -RT				Undet	Middle-Aged -RT				Undet
Infant +RT	F	5m	0.417	40.000	Middle-Aged +RT	F	56y	56.000	38.425
Infant -RT				Undet	Middle-Aged -RT				Undet
Toddler +RT	M	10m	0.833	40.000	Middle-Aged +RT	M	61y	61.000	38.577
Toddler -RT				Undet	Middle-Aged -RT				Undet
Toddler +RT	F	19m	1.583	37.562	Elderly +RT	M	66y	66.000	34.822
Toddler -RT				Undet	Elderly -RT				Undet
Child +RT	M	4y	4.000	40.000	Elderly +RT	F	71y	71.000	37.476
Child -RT				Undet	Elderly -RT				Undet
Child +RT	F	12y	12.000	39.208	Elderly +RT	M	76y	76.000	36.217
Child -RT				Undet	Elderly -RT				Undet
Juvenile +RT	M	15y	15.000	35.557	Elderly +RT	F	81y	81.000	34.500
Juvenile -RT				Undet	Elderly -RT				Undet
Juvenile +RT	M	18y	18.000	35.813	Elderly +RT	F	84y	84.000	35.280
Juvenile -RT				Undet	Elderly -RT				Undet
Adult +RT	M	22y	22.000	37.572	Elderly +RT	M	86y	86.000	36.229
Adult -RT				28.176	Elderly -RT				Undet
Adult +RT	M	27y	27.000	39.987	Elderly +RT	F	91y	91.000	36.459
Adult -RT				Undet	Elderly -RT				Undet
Adult +RT	M	35y	35.000	39.063	Elderly +RT	M	92y	92.000	36.382
Adult -RT				Undet	Elderly -RT				Undet
Adult +RT	M	40y	40.000	35.493	DNA				
Adult -RT				Undet	DNA				
Adult +RT	M	43y	43.000	36.318	NTC				
Adult -RT				Undet	NTC				

Table 14: IGFBP3 Real-Time PCR Duplex Delta Ct Results.

Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value	Sample #	Age	Ct (GOI)	Ct (S15)	dCt Value
Newborn +RT	1h	40.000	35.618	-4.382	Adult +RT	36y	36.329	35.712	-0.617
Newborn +RT	1h	40.000	40.000	0.000	Adult +RT	36y	36.693	36.955	0.262
Newborn +RT	2d	40.000	37.407	-2.593	Adult +RT	38y	35.831	34.602	-1.229
Newborn +RT	8d	36.605	34.137	-2.468	Adult +RT	40y	36.649	35.890	-0.759
Newborn +RT	17d	40.000	35.880	-4.120	Adult +RT	40y	34.720	34.943	0.223
Newborn +RT	1m	40.000	36.377	-3.623	Adult +RT	43y	37.365	35.865	-1.500
Newborn +RT	1m	40.000	40.000	0.000	Adult +RT	43y	38.017	39.724	1.707
Newborn +RT	2m	40.000	35.188	-4.812	Adult +RT	45y	35.068	34.117	-0.951
Newborn +RT	3m	37.728	35.044	-2.684	Elderly +RT	46y	35.485	36.726	1.241
Infant +RT	4m	38.677	34.410	-4.267	Elderly -RT	47y	39.947	40.000	0.053
Infant +RT	5m	40.000	34.060	-5.940	Middle-Age +RT	47y	36.340	34.229	-2.111
Infant +RT	7m	38.164	34.523	-3.641	Middle-Age +RT	49y	39.055	40.000	0.945
Infant +RT	9m	40.000	37.713	-2.287	Middle-Age +RT	51y	36.384	36.170	-0.214
Toddler +RT	10m	40.000	35.294	-4.706	Middle-Age +RT	53y	40.000	36.422	-3.578
Toddler +RT	14m	40.000	35.862	-4.138	Middle-Age +RT	53y	34.370	36.711	2.341
Toddler +RT	18m	40.000	35.584	-4.416	Middle-Age +RT	56y	36.037	37.489	1.452
Toddler +RT	21m	40.000	35.930	-4.070	Middle-Age +RT	56y	36.392	39.560	3.168
Toddler +RT	2y	40.000	35.701	-4.299	Middle-Age +RT	57y	35.489	36.810	1.321
Toddler +RT	3y	38.164	35.136	-3.028	Middle-Age +RT	58y	38.215	35.917	-2.298
Child +RT	4y	40.000	32.741	-7.259	Middle-Age +RT	60y	37.188	34.025	-3.163
Child +RT	4y	40.000	36.483	-3.517	Middle-Age +RT	61y	37.657	39.238	1.581
Child +RT	5y	38.097	33.272	-4.825	Middle-Age +RT	63y	37.585	39.318	1.733
Child +RT	6y	40.000	36.590	-3.410	Middle-Age +RT	63y	39.234	33.748	-5.486
Child +RT	8y	40.000	35.451	-4.549	Elderly +RT	65y	36.314	35.654	-0.660
Child +RT	9y	40.000	36.488	-3.512	Elderly +RT	66y	35.238	39.637	4.399
Child +RT	9y	38.375	36.635	-1.740	Elderly +RT	68y	34.411	37.674	3.263
Child +RT	12y	40.000	36.751	-3.249	Elderly +RT	68y	35.321	32.247	-3.074
Child +RT	12y	39.207	35.559	-3.648	Elderly +RT	69y	35.680	35.258	-0.422
Juvenile +RT	13y	40.000	32.970	-7.030	Elderly +RT	71y	37.262	40.000	2.738
Juvenile +RT	14y	36.370	34.298	-2.072	Elderly +RT	71y	36.505	35.812	-0.693
Juvenile +RT	14y	34.154	36.280	2.126	Elderly +RT	72y	37.797	37.428	-0.369
Juvenile +RT	15y	40.000	35.587	-4.413	Elderly +RT	74y	37.054	37.104	0.050
Juvenile +RT	15y	36.442	33.560	-2.882	Elderly +RT	76y	34.124	35.432	1.308
Juvenile +RT	16y	40.000	35.428	-4.572	Elderly +RT	76y	36.540	38.447	1.907
Juvenile +RT	16y	37.381	34.729	-2.652	Elderly +RT	79y	37.995	40.000	2.005
Juvenile +RT	17y	40.000	35.018	-4.982	Elderly +RT	80y	37.482	36.158	-1.324
Juvenile +RT	18y	34.484	31.980	-2.504	Elderly +RT	81y	35.630	37.650	2.020
Adult +RT	19y	35.387	34.980	-0.407	Elderly +RT	83y	40.000	35.812	-4.188
Adult +RT	21y	40.000	38.267	-1.733	Elderly +RT	84y	34.619	34.887	0.268
Adult +RT	23y	36.483	35.140	-1.343	Elderly +RT	84y	35.079	35.859	0.780
Adult +RT	24y	35.216	37.270	2.054	Elderly +RT	86y	35.056	34.884	-0.172
Adult +RT	25y	36.657	35.134	-1.523	Elderly +RT	89y	35.854	34.418	-1.436
Adult +RT	27y	38.964	35.944	-3.020	Elderly +RT	89y	34.860	34.106	-0.754
Adult +RT	29y	35.624	30.368	-5.256	Elderly +RT	91y	35.274	35.345	0.071
Adult +RT	29y	37.118	35.717	-1.401	Elderly +RT	92y	36.073	34.559	-1.514
Adult +RT	31y	35.212	34.600	-0.612	Elderly +RT	102y	31.403	22.654	-8.749
Adult +RT	35y	39.757	36.147	-3.610	DNA		50.000	50.000	0.000
Adult +RT	35y	36.306	36.550	0.244	NTC		50.000	50.000	0.000

Table 15: IGFBP3 Triplicate qPCR Results.

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Newborn +RT	1h	50.000	0.000	38.570	0.522	-11.430	0.522
Newborn +RT	1h	39.407	1.411	34.988	0.491	-4.419	1.871
Newborn +RT	1h	50.000	0.000	33.631	0.202	-16.369	0.202
Newborn +RT	1h	50.000	0.000	35.938	1.078	-14.062	1.078
Newborn +RT	1d	38.087	0.862	34.066	0.395	-4.021	0.590
Newborn +RT	2d	50.000	0.000	35.107	0.235	-14.893	0.235
Newborn +RT	8d	39.029	0.176	35.840	0.235	-3.189	0.233
Newborn +RT	17d	50.000	0.000	35.502	0.235	-14.498	0.235
Newborn +RT	1m	42.714	1.414	35.418	0.436	-7.296	0.993
Newborn +RT	1m	50.000	0.000	33.628	0.010	-16.372	0.010
Newborn +RT	2m	50.000	0.000	34.336	0.547	-15.664	0.547
Newborn +RT	3m	38.782	0.591	35.763	0.347	-3.018	0.284
Newborn +RT	3m	50.000	0.000	36.902	0.810	-13.098	0.810
Infant +RT	4m	38.146	0.690	35.441	0.444	-2.705	0.250
Infant +RT	4m	38.012	2.273	34.384	1.587	-3.628	1.165
Infant +RT	5m	50.000	0.000	34.733	0.256	-15.267	0.256
Infant +RT	5m	50.000	0.000	35.547	0.848	-14.453	0.848
Infant +RT	6m	50.000	0.000	35.691	0.396	-14.309	0.396
Infant +RT	7m	39.634	0.560	34.934	0.589	-4.701	0.064
Infant +RT	7m	38.247	0.439	35.108	0.201	-3.139	0.593
Infant +RT	7m	49.245	1.307	35.536	0.104	-13.710	1.405
Infant +RT	8m	50.000	0.000	34.021	0.317	-15.979	0.317
Infant +RT	8m	50.000	0.000	37.711	0.589	-12.289	0.589
Infant +RT	8m	37.256	0.360	33.955	0.295	-3.300	0.643
Toddler +RT	10m	39.289	0.168	35.597	0.281	-3.692	0.113
Toddler +RT	14m	50.000	0.000	36.456	1.170	-13.544	1.170
Toddler +RT	14m	50.000	0.000	34.656	0.533	-15.344	0.533
Toddler +RT	15m	50.000	0.000	37.107	0.709	-12.893	0.709
Toddler +RT	18m	38.590	0.978	35.428	0.252	-3.161	1.011
Toddler +RT	19m	39.169	0.508	34.943	0.505	-4.226	0.294
Toddler +RT	2y	50.000	0.000	36.797	0.952	-13.203	0.952
Toddler +RT	2.8y	50.000	0.000	34.670	0.920	-15.330	0.920
Toddler +RT	3y	39.224	0.563	35.263	0.209	-3.961	0.443
Child +RT	4y	38.398	0.431	35.682	0.764	-2.716	0.333
Child +RT	4y	50.000	0.000	34.535	0.607	-15.465	0.607
Child +RT	5y	36.425	0.346	32.738	0.333	-3.686	0.017
Child +RT	6y	50.000	0.000	36.892	0.147	-13.108	0.147
Child +RT	8y	36.771	0.984	35.901	0.392	-0.870	1.110
Child +RT	9y	38.757	0.305	35.561	0.584	-3.195	0.471
Child +RT	9y	38.836	0.541	38.401	0.628	-0.435	0.097
Child +RT	12y	37.708	0.780	35.979	0.163	-1.729	0.937
Child +RT	12y	38.628	0.655	36.171	0.175	-2.457	0.829
Child +RT	12y	38.528	1.005	35.136	0.359	-3.393	0.947

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Juvenile +RT	13y	38.718	0.405	35.536	0.215	-3.181	0.485
Juvenile +RT	14y	37.083	0.963	34.896	0.280	-2.187	0.961
Juvenile +RT	14y	34.963	0.298	34.661	0.608	-0.302	0.548
Juvenile +RT	14y	36.474	0.214	35.355	0.547	-1.120	0.590
Juvenile +RT	15y	37.191	1.536	32.871	0.527	-4.320	1.690
Juvenile +RT	15y	35.983	0.621	33.838	1.148	-2.145	0.896
Juvenile +RT	16y	36.094	0.127	32.933	0.249	-3.161	0.326
Juvenile +RT	16y	37.115	0.249	35.348	0.344	-1.767	0.440
Juvenile +RT	17y	50.000	0.000	36.925	0.374	-13.075	0.374
Juvenile +RT	17y	50.000	0.000	40.366	0.583	-9.634	0.583
Juvenile +RT	17y	39.957	1.932	36.517	0.362	-3.440	1.821
Juvenile +RT	18y	35.116	0.113	32.522	0.357	-2.594	0.259
Juvenile +RT	18y	38.153	2.200	35.351	0.869	-2.802	1.652
Adult +RT	19y	37.441	0.137	37.079	0.181	-0.361	0.068
Adult +RT	19y	36.567	0.697	35.985	0.287	-0.581	0.490
Adult +RT	21y	50.000	0.000	37.798	0.671	-12.202	0.671
Adult +RT	21y	38.887	1.142	35.585	1.127	-3.301	1.658
Adult +RT	22y	35.130	0.685	37.705	1.089	2.576	1.566
Adult +RT	22y	35.895	0.468	35.680	0.428	-0.215	0.825
Adult +RT	23y	37.117	0.578	36.050	0.558	-1.067	0.303
Adult +RT	24y	34.147	0.329	33.977	0.432	-0.171	0.220
Adult +RT	25y	38.488	0.851	37.699	0.233	-0.789	1.014
Adult +RT	26y	33.317	0.283	34.736	0.171	1.420	0.152
Adult +RT	26y	38.590	0.824	37.115	0.292	-1.475	0.692
Adult +RT	27y	37.427	0.733	35.920	0.285	-1.507	0.603
Adult +RT	29y	35.950	0.550	35.067	0.675	-0.883	0.987
Adult +RT	29y	35.837	0.469	35.680	0.049	-0.157	0.420
Adult +RT	35y	37.977	0.651	36.385	0.898	-1.592	1.174
Adult +RT	35y	35.787	0.223	36.768	1.040	0.981	1.050
Adult +RT	36y	36.175	0.137	36.608	0.184	0.433	0.227
Adult +RT	36y	36.768	0.607	35.465	0.234	-1.304	0.382
Adult +RT	38y	35.467	0.546	33.341	0.268	-2.126	0.685
Adult +RT	38y	36.300	0.164	36.247	0.139	-0.052	0.156
Adult +RT	38y	34.283	0.648	35.318	0.265	1.035	0.457
Adult +RT	40y	37.911	1.075	34.966	0.181	-2.945	1.086
Adult +RT	40y	34.112	0.309	36.223	1.173	2.111	0.975
Adult +RT	43y	36.559	0.610	36.939	0.728	0.380	0.588
Adult +RT	45y	34.969	0.207	33.670	1.421	-1.298	1.401
Adult +RT	45y	35.541	0.555	34.575	0.393	-0.966	0.905
Adult +RT	45y	33.149	0.233	34.542	0.573	1.393	0.343

Sample	Age	Average Ct GOI	SD GOI	Average Ct S15	SD S15	Average dCt	SD dCt
Middle-Age +RT	46y	36.191	0.863	36.864	0.672	0.673	1.176
Middle-Age +RT	47y	35.795	0.384	36.751	1.103	0.956	0.770
Middle-Age +RT	47y	37.376	0.893	36.643	0.439	-0.733	0.826
Middle-Age +RT	51y	36.298	0.356	35.528	0.021	-0.770	0.347
Middle-Age +RT	53y	50.000	0.000	41.367	0.611	-8.633	0.611
Middle-Age +RT	53y	34.138	0.649	35.843	1.067	1.704	0.680
Middle-Age +RT	56y	38.107	0.272	37.150	0.056	-0.957	0.292
Middle-Age +RT	56y	36.877	0.481	35.947	0.167	-0.930	0.598
Middle-Age +RT	57y	36.000	0.407	35.856	0.328	-0.143	0.265
Middle-Age +RT	58y	37.855	0.910	36.846	0.201	-1.009	0.921
Middle-Age +RT	59y	36.226	0.631	36.343	0.254	0.117	0.779
Middle-Age +RT	60y	36.856	0.177	35.514	0.465	-1.342	0.336
Middle-Age +RT	61y	36.764	0.713	36.297	1.603	-0.467	1.237
Middle-Age +RT	61y	36.284	0.398	35.662	1.467	-0.622	1.096
Middle-Age +RT	63y	38.499	0.686	37.161	0.210	-1.338	0.728
Middle-Age +RT	63y	37.668	0.782	35.015	0.248	-2.653	0.740
Elderly +RT	65y	38.641	0.486	37.875	0.419	-0.767	0.901
Elderly +RT	66y	36.362	0.200	40.077	0.515	3.715	0.316
Elderly +RT	66y	35.700	1.221	37.278	1.057	1.578	0.850
Elderly +RT	68y	35.378	0.626	37.045	0.518	1.666	0.771
Elderly +RT	68y	35.210	0.656	35.815	0.410	0.605	0.764
Elderly +RT	69y	36.190	0.752	35.968	0.287	-0.222	0.502
Elderly +RT	71y	50.000	0.000	37.466	0.842	-12.534	0.842
Elderly +RT	71y	37.558	1.188	36.608	0.253	-0.950	1.342
Elderly +RT	71y	37.155	1.963	35.963	0.688	-1.192	2.651
Elderly +RT	72y	37.689	0.424	37.167	0.815	-0.522	1.162
Elderly +RT	74y	36.982	1.253	38.339	0.679	1.357	1.009
Elderly +RT	76y	34.893	0.582	36.091	1.359	1.198	1.179
Elderly +RT	76y	36.849	1.373	36.681	0.287	-0.168	1.105
Elderly +RT	79y	37.504	0.614	37.753	0.983	0.249	0.576
Elderly +RT	80y	37.013	0.685	35.665	0.108	-1.347	0.579
Elderly +RT	81y	33.966	0.211	35.334	0.309	1.368	0.217
Elderly +RT	84y	35.291	0.272	35.880	0.269	0.588	0.480
Elderly +RT	84y	33.947	0.427	34.742	0.910	0.796	1.288
Elderly +RT	86y	35.380	0.293	34.175	0.319	-1.205	0.074
Elderly +RT	89y	36.909	0.505	35.011	0.656	-1.897	0.486
Elderly +RT	89y	35.134	0.375	35.417	1.039	0.282	1.388
Elderly +RT	91y	36.501	0.523	36.352	0.582	-0.149	1.002
Elderly +RT	92y	36.122	0.658	34.886	0.235	-1.236	0.824
Elderly +RT	102y	36.158	0.876	35.149	0.573	-1.009	1.140

Table 16: Primer, Probe Sequences and Expected Product Sizes for the RT-PCR Newborn Assays.

RT-PCR Assay	Primer Sequences 5'→ 3'	DNA (bp)	RNA (bp)
S15			
Forward	5’ TTC-CGC-AAG-TTC-ACC-TAC-C 3’	361	361
Reverse	5’ CGG-GCC-GGC-CAT-GCT-TTA-CG 3’		
GNAS			
Forward	5’ AAG-ATC-GAC-GTG-ATC-AAG-CA 3’	855	371
Reverse	5’ CCA-GCA-AGG-ACT-TTC-TCA-GC 3’		
HBG			
Forward	5’ GTG-GAT-CCT-GAG-AAC-TTC-AA 3’	1040	154
Reverse	5’ GAG-CTC-AGT-GGT-ATC-TGG-AG 3’		
HBG1			
Forward	5’ ACT-TCC-TTG-GGA-GAT-GCC-AC 3’	1157	277
Reverse	5’ AAA-GCC-TAT-CCT-TGA-AAG-CTC-TGA 3’		
HBG2			
Forward	5’ ACT-TCC-TTG-GGA-GAT-GCC-AT 3’	1160	274
Reverse	5’ GCC-TAT-CCT-TGA-AAG-CTC-TGC 3’		
HBG1n1			
Forward	5’ GAA-AGC-TCT-GAA-TCA-TCC-AGG-TG 3’ a	0	207
Reverse	5’ GGG-CAA-GGT-GAA-TGT-GGA-AG 3’		
HBG1n2			
Forward	5’ AGT-GAG-CTC-AGT-GGC-ATC-TC 3' a	0	190
Reverse	5’ GGG-CAA-GGT-GAA-TGT-GGA-AG 3’		
HBG2n2			
Forward	5’ CTG-GAG-GAC-AGG-GCA-AAG-G 3’ a	0	225
Reverse	5’ GGG-CAA-GGT-GAA-TGT-GGA-AG 3’		
HBG2n3			
Forward	5’ GGC-AGT-GAG-CTC-AGT-GCA-GTT-C 3’ a	0	161
Reverse	5’ CAG-CTT-TGG-CAA-CCT-GTC-CT 3’		

a Underlined sequence identifies the location of the newborn hemoglobin isoform breakpoints

Table 17: Real-Time PCR primer and probe sequences for Forensic Newborn Identification.

qRT-PCR Assay	Primer & Probe Sequences 5'→ 3'
S15	
Forward	5' CCA-AAG-CGA-TCT-CTT-CTG-AGG-AT 3'
Reverse	5' ACG-CCG-CGG-TAG-GTG-AA 3'
Probe	VIC CGG-CAA-GAT-GGC-AGA-AGT-AGA-GCA-GAA MGBNFQ b
HBG1n1	
Forward	5' GAA-AGC-TCT-GAA-TCA-TCC-AGG-TG 3' a
Reverse	5' AGT-CAA-GGC-ACA-TGG-CAA-GAA-G 3'
Probe	6FAM TTT-GTG-GCA-TCT-CCC-AAG-GAA-GTC-AGC MGBNFQ b
HBG2n3	
Forward	5' GCA-GTG-AGC-TCA-GTG-CAG-TTC 3' a
Reverse	5' TTC-CTT-GGG-AGA-TGC-CAT-AAA 3'
Probe	6FAM CAA-AGG-TGC-CCT-TGA-GAT-CAT-CCA-GG MGBNFQ b

a Underlined sequence identifies the location of the newborn hemoglobin isoform breakpoints

b MGBNFQ, minor groove binding non-fluorescent quencher

Table 18: Biological Age Specificity Results for the Two Newborn Duplex qPCR Assays.

		Assay Results					
		≤4 months			<24 hours		
Biological Age	n=	(+/+)[a]	(+/–)[b]	(–/–)[c]	(+/+)[a]	(+/–)[b]	(–/–)[c]
<24 hours	10	10	-	-	10	-	-
1 day – 1 month	19	19	-	-	3	14	2
2 months – 4 months	22	17	4	1	-	1	21
5 months – 3 years	37	1	2	34	-	2	35
4 years – 18 years	20	-	-	20	-	-	20
19 years – 92 years	24	-	-	24	-	-	24

[a] represents two positive delta Ct values (+/+)
[b] represents one positive delta Ct value and one negative delta Ct value (+/–)
[c] represents two negative delta Ct values (–/–)

Table 19: Sensitivity Data for ≤ 4 Month Newborn Duplex Assays.

Input RNA (picograms)	NEWBORN			ADULT		
	Ct HBG1n	Ct S15	dCt (S15-HBG1n)	Ct HBG1n	Ct S15	dCt (S15-HBG1n)
1000	28.460	31.336	+2.876	40.000	33.665	-6.336
750	28.132	31.259	+3.128	40.000	34.343	-5.657
500	28.878	31.881	+3.003	40.000	34.805	-5.195
250	31.132	33.190	+2.058	40.000	37.173	-2.827
100	32.873	35.084	+2.211	40.000	37.625	-2.376
50	34.116	37.213	+3.097	40.000	38.505	-1.496
25	35.732	37.576	+1.844	40.000	39.813	-0.187
10	35.285	38.146	+2.862	40.000	40.000	0
5	34.896	38.732	+3.836	40.000	40.000	0
1	40.000	40.000	0	40.000	40.000	0
	Ct HBG2n	Ct S15	dCt (S15-HBG2n)	Ct HBG2n	Ct S15	dCt (S15-HBG2n)
1000	29.849	37.829	+7.981	40.000	37.778	-2.222
750	28.624	36.094	+7.470	40.000	37.417	-2.584
500	29.710	35.589	+5.879	40.000	38.152	-1.849
250	30.602	36.095	+5.494	40.000	37.931	-2.069
100	30.165	38.382	+8.217	40.000	36.569	-3.432
50	32.143	37.720	+5.577	40.000	38.619	-1.381
25	37.952	39.295	+1.343	40.000	40.000	0
10	35.441	40.000	+4.560	40.000	40.000	0
5	38.616	40.000	+1.385	40.000	40.000	0
1	39.787	40.000	+0.213	40.000	40.000	0

Ct, cycle threshold; dCt, delta cycle threshold

Table 20: Sensitivity Data for < 24 Hour Newborn Duplex Assays.

Input RNA (picograms)	NEWBORN			ADULT		
	Ct HBG1n	Ct S15	dCt (S15-HBG1n)	Ct HBG1n	Ct S15	dCt (S15-HBG1n)
1000	32.651	34.466	+1.815	39.101	34.849	-4.252
800	32.810	34.504	+1.694	39.196	35.075	-4.121
600	32.900	34.631	+1.732	39.261	35.021	-4.240
400	33.892	35.531	+1.639	39.549	35.596	-3.953
200	34.053	35.414	+1.362	39.543	35.915	-3.628
100	35.664	37.211	+1.547	40.000	37.302	-2.698
50	36.577	37.809	+1.232	40.000	38.658	-1.342
25	37.382	38.430	+1.049	39.966	39.132	-0.834
10	38.663	39.824	+1.162	37.898	39.640	+1.742
5	39.635	40.000	+0.365	37.863	37.972	+0.109
1	40.000	40.000	0	40.000	40.000	0
	Ct HBG2n	Ct S15	dCt (S15-HBG2n)	Ct HBG2n	Ct S15	dCt (S15-HBG2n)
1000	28.166	35.389	+7.224	36.622	35.741	-0.881
800	29.007	34.724	+5.717	36.561	35.298	-1.263
600	29.132	34.921	+5.788	37.188	35.225	-1.963
400	30.959	35.381	+4.421	38.406	35.665	-2.741
200	32.203	36.192	+3.989	39.872	36.940	-2.933
100	33.191	37.636	+4.444	40.000	37.599	-2.401
50	34.441	38.250	+3.809	40.000	38.415	-1.585
25	31.307	34.646	+3.339	40.000	40.000	0
10	37.177	39.854	+2.677	40.000	40.000	0
5	38.467	39.969	+1.502	40.000	40.000	0
1	39.779	40.000	+0.221	40.000	40.000	0

Ct, cycle threshold; dCt, delta cycle threshold

Table 21: Telomere Real-time PCR and STELA primer, probe, and linker sequences.

Assay	Primer, Probe, & Linker Sequences
Real-Time PCR	
36B4u	5' CAG-CAA-GTG-GGA-AGG-TGT-AAT-CC 3' [a]
36B4d	5' CCC-ATT-CTA-TCA-TCA-ACG-GGT-ACA-A 3' [a]
tel 1	5' GGT-TTT-TGA-GGG-TGA-GGG-TGA-GGG-TGA-GGG-TGA-GGG-T 3' [a]
tel 2	5' TCC-CGA-CTA-TCC-CTA-TCC-CTA-TCC-CTA-TCC-CTA-TCC-CTA 3' [a]
tel 3	5' VIC-CCC-TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC-TAA-C-TAMRA 3'
tel 4	5' GGT-TTT-TGA-GGG-TGA-GGG-TGA-GGG-T 3'
tel 5	5' TCC-CGA-CTA-TCC-CTA-TCC-CTA-TCC-CTA 3'
tel 6	5' VIC-CCC-TAA-CCC-TAA-CCC-TAA-C-TAMRA 3'
STELA	
Telorette 1	5' TGC-TCC-GTG-CAT-CTG-GCA-TCC-CCT-AAC 3' [b]
Telorette 2	5' TGC-TCC-GTG-CAT-CTG-GCA-TCT-AAC-CCT 3' [b]
Telorette 3	5' TGC-TCC-GTG-CAT-CTG-GCA-TCC-CTA-ACC 3' [b]
Telorette 4	5' TGC-TCC-GTG-CAT-CTG-GCA-TCC-TAA-CCC 3' [b]
Telorette 5	5' TGC-TCC-GTG-CAT-CTG-GCA-TCA-ACC-CTA 3' [b]
Telorette 6	5' TGC-TCC-GTG-CAT-CTG-GCA-TCA-CCC-TAA 3' [b]
TelTail	5' TGC-TCC-GTG-CAT-CTG-GCA-TC 3' [b]
XpYpE2	5' TTG-TCT-CAG-GGT-CCT-AGT-G 3' [b]

[a] Cawthon R, Telomere measurement by quantitative PCR, Nucleic Acids Research, 2002, 30 (10).
[b] Baird D et al., Extensive allelic variation and ultrashort telomeres in senescent human cells, Nature Genetics, 2003, 33.

Table 22: SYBR Green I Real-time PCR Amplification Results for Telomere Delta Ct Determination.

DNA Sample	Total DNA added to reaction (ng)	Reaction volume (uL)	Average Single Copy Gene Ct value (S)*	Average Telomere Ct value (T)	Delta Ct Value (S – T)	±SD
Standards						
STD1	50.0	25.0	22.405	20.785	1.620	0.085
STD2	30.0	25.0	23.270	21.780	1.490	0.000
STD3	17.7	25.0	24.085	22.850	1.235	0.092
STD4	10.6	25.0	25.045	24.170	0.875	0.064
STD5	6.3	25.0	26.065	24.775	1.290	0.014
Unknowns						
1-hour	20.0	25.0	27.520	24.915	2.605	0.148
1-hour	20.0	25.0	26.785	23.650	3.135	0.290
4-years	20.0	25.0	27.320	25.240	2.080	0.184
9-years	20.0	25.0	32.240	29.395	2.845	0.587
14-years	20.0	25.0	27.490	24.570	2.920	0.071
17-years	20.0	25.0	28.765	25.455	3.310	0.127
45-years	20.0	25.0	27.410	25.360	2.050	0.198
47-years	20.0	25.0	27.480	24.555	2.925	0.134
60-years	20.0	25.0	27.955	25.705	2.250	0.028
63-years	20.0	25.0	26.580	23.745	2.835	0.120
89-years	20.0	25.0	28.630	25.555	3.075	0.290
91-years	20.0	25.0	27.900	24.540	3.360	0.198

*The single copy gene is the acidic ribosomal phosphoprotein PO gene, 36B4

Table 23: Quantitative Telomere Amplification using the TaqMan real-time PCR platform.

DNA Sample	Total DNA added to reaction (ng)	Reaction volume (uL)	tel 1, 2, 3 Ct value	tel 4, 5, 6 Ct value
1-hour	10.0	25.0	9.992	6.018
1-hour	10.0	25.0	9.587	5.872
1-hour	10.0	25.0	9.945	6.911
1-hour	10.0	25.0	10.811	6.189
2-years	10.0	25.0	10.096	5.348
4-years	10.0	25.0	10.098	6.391
9-years	10.0	25.0	11.243	6.805
12-years	10.0	25.0	9.115	5.261
14-years	10.0	25.0	9.040	6.223
16-years	10.0	25.0	9.307	5.901
17-years	10.0	25.0	9.760	6.321
31-years	10.0	25.0	8.438	5.560
45-years	10.0	25.0	9.397	6.038
47-years	10.0	25.0	9.215	6.058
60-years	10.0	25.0	9.616	5.822
63-years	10.0	25.0	9.579	6.298
86-years	10.0	25.0	9.893	5.958
89-years	10.0	25.0	9.704	6.645
91-years	10.0	25.0	9.687	6.244

APPENDIX C: AFFYMETRIX COMPARISONS

Table 24: Affymetrix Comparisons.

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
216317_x_at	1789.62	132.97	13.46	0.0426	RHCE
224734_at	1204.95	93.34	9.41	0.0185	HMGB1
219083_at	1866.19	205.48	9.08	0.0195	FLJ10539
209066_x_at	981.98	85.46	7.67	0.0152	UQCRB
239247_at	136.72	1037.14	7.59	0.0320	
212855_at	964.64	23.36	7.54	0.0124	KIAA0276
227224_at	1001.40	134.83	7.43	0.0084	FLJ10244
218515_at	942.25	119.00	7.36	0.0311	C21orf66
222142_at	925.38	97.40	7.23	0.0203	CYLD
202174_s_at	1084.95	151.12	7.18	0.0462	PCM1
223391_at	916.99	35.87	7.16	0.0097	SGPP1
225611_at	915.74	112.03	7.15	0.0039	KIAA0303
242930_at	85.70	905.94	7.08	0.0138	OSGEP
230448_at	123.38	901.11	7.04	0.0297	MGC15523
216502_at	114.54	895.47	7.00	0.0410	FLJ12671
218437_s_at	875.04	76.03	6.84	0.0495	LZTFL1
201851_at	78.33	868.76	6.79	0.0041	SH3GL1
217094_s_at	1404.47	212.11	6.62	0.0155	ITCH
226561_at	849.01	129.13	6.57	0.0440	LOC285086
204647_at	157.76	1021.12	6.47	0.0227	HOMER3
215613_at	91.79	821.16	6.42	0.0084	ADAM12
203202_at	801.70	71.74	6.26	0.0021	HRB2
235196_at	799.74	62.44	6.25	0.0113	C1orf28
223590_at	794.40	45.64	6.21	0.0438	DKFZp434I1610
1562671_s_at	153.05	949.05	6.20	0.0066	
229285_at	787.24	20.48	6.15	0.0371	RNASEL
236994_at	774.09	69.02	6.05	0.0022	na
202209_at	771.57	126.59	6.03	0.0427	LSM3
209798_at	1885.92	318.29	5.93	0.0196	NPAT
225295_at	911.10	157.48	5.79	0.0426	KIAA1265
227982_at	736.97	85.71	5.76	0.0428	SLA/LP
204645_at	827.41	144.92	5.71	0.0258	CCNT2
228366_at	806.95	142.11	5.68	0.0137	PPA2
224728_at	874.62	163.82	5.34	0.0343	ATPAF1
227548_at	874.07	164.00	5.33	0.0407	LOC51240
1557706_at	136.94	728.38	5.32	0.0176	
1564257_at	157.50	825.01	5.24	0.0121	ACTA2
1557873_at	269.76	1391.05	5.16	0.0449	LOC153218

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
233987_at	55.32	658.47	5.14	0.0017	TFAP2BL1
212938_at	117.35	658.04	5.14	0.0032	COL6A1
228019_s_at	653.62	79.04	5.11	0.0177	MRPS18C
1569566_at	41.97	642.02	5.02	0.0336	na
239479_x_at	639.15	117.66	4.99	0.0127	
208520_at	155.17	769.23	4.96	0.0216	OR10H3
210147_at	28.85	632.39	4.94	0.0491	ART3
215226_at	131.10	642.86	4.90	0.0278	
235086_at	624.73	95.11	4.88	0.0122	THBS1
201369_s_at	123.26	623.64	4.87	0.0319	ZFP36L2
223328_at	622.08	111.87	4.86	0.0221	SVH
230029_x_at	620.20	43.82	4.85	0.0114	KIAA2024
206314_at	620.15	60.28	4.84	0.0102	ZFP
242550_at	79.79	617.30	4.82	0.0488	
226807_at	610.45	59.68	4.77	0.0055	ZFP1
207735_at	85.33	602.05	4.70	0.0208	RNF125
201663_s_at	645.42	139.56	4.62	0.0056	SMC4L1
212568_s_at	590.08	70.09	4.61	0.0145	DLAT
219215_s_at	85.57	590.04	4.61	0.0222	SLC39A4
218970_s_at	1047.18	227.84	4.60	0.0481	CGI-32
235437_at	103.38	588.16	4.59	0.0099	
218599_at	85.33	586.81	4.58	0.0088	REC8
207404_s_at	190.30	869.29	4.57	0.0492	HTR1E
212243_at	958.18	211.50	4.53	0.0007	GRINL1A
212567_s_at	90.46	578.66	4.52	0.0105	MAP4
37201_at	40.26	577.24	4.51	0.0293	ITIH4
229798_s_at	317.41	1420.69	4.48	0.0247	BRI3
225455_at	1139.63	256.34	4.45	0.0379	STAF42
212805_at	1513.82	341.03	4.44	0.0427	KIAA0367
219354_at	77.36	567.91	4.44	0.0010	FLJ11078
222559_s_at	563.43	59.38	4.40	0.0137	P15RS
222204_s_at	562.44	95.30	4.39	0.0237	RRN3
1554568_at	45.08	560.41	4.38	0.0370	DNCL2A
1559654_s_at	202.69	874.37	4.31	0.0332	
243173_at	71.86	549.54	4.29	0.0482	MGC57793
227934_at	2788.28	649.46	4.29	0.0213	
244076_at	103.96	549.35	4.29	0.0244	KIAA2022
204955_at	45.54	549.19	4.29	0.0032	SRPX
226438_at	1196.42	279.00	4.29	0.0097	SNTB1
220992_s_at	548.68	26.10	4.29	0.0082	C1orf25
233943_x_at	96.40	547.60	4.28	0.0238	FAPP2

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
1561089_at	147.37	628.85	4.27	0.0149	na
209512_at	544.11	58.44	4.25	0.0045	MGC10940
236281_x_at	107.90	543.08	4.24	0.0293	HTR7
231833_at	92.49	541.95	4.23	0.0072	MGC20460
235738_at	107.09	538.81	4.21	0.0266	
227015_at	202.32	849.06	4.20	0.0298	LOC57168
227836_at	535.18	124.80	4.18	0.0141	MGC14595
1566001_at	335.48	1401.89	4.18	0.0337	
202260_s_at	123.58	520.58	4.07	0.0416	STXBP1
201165_s_at	1571.61	386.86	4.06	0.0085	PUM1
209919_x_at	218.18	880.48	4.04	0.0165	GGT1
226437_at	91.68	515.16	4.02	0.0472	LOC90522
232647_at	132.06	531.43	4.02	0.0181	MGC39650
228381_at	2702.31	672.23	4.02	0.0115	ATF7IP2
219009_at	115.95	513.11	4.01	0.0057	C14orf93
222601_at	510.72	29.18	3.99	0.0002	FLJ10808
218414_s_at	242.57	967.34	3.99	0.0236	NDE1
219118_at	94.83	510.35	3.99	0.0036	FKBP11
241342_at	595.22	149.73	3.98	0.0131	LOC157378
231832_at	995.25	251.70	3.95	0.0383	TUWD12
222156_x_at	1039.87	264.37	3.93	0.0472	CPR8
230466_s_at	2484.72	631.85	3.93	0.0470	
230776_at	498.40	78.01	3.89	0.0119	KIAA1917
1553523_at	147.88	573.01	3.87	0.0420	NALP14
229335_at	128.33	496.30	3.87	0.0377	TSLL2
231190_at	126.88	490.87	3.83	0.0402	na
215135_at	294.12	1105.83	3.76	0.0042	DNPEP
203341_at	480.27	40.37	3.75	0.0028	CBF2
239532_at	198.87	742.54	3.73	0.0173	na
216300_x_at	72.98	468.70	3.66	0.0283	RARA
224827_at	468.52	77.36	3.66	0.0117	DC-UbP
1557845_at	63.77	468.22	3.66	0.0227	
226687_at	1269.07	348.01	3.65	0.0294	FNBP3
236712_at	90.67	460.73	3.60	0.0346	
213683_at	517.40	144.02	3.59	0.0216	FACL6
1562137_at	111.12	459.36	3.59	0.0183	
225904_at	458.48	48.01	3.58	0.0022	
213256_at	1931.33	540.03	3.58	0.0338	MGC48332
242003_at	164.38	585.70	3.56	0.0496	
219236_at	307.30	1084.54	3.53	0.0027	FLJ22672
1557749_at	142.81	502.01	3.52	0.0180	DKFZp762C186

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
227109_at	449.88	61.30	3.51	0.0087	CYP2R1
242783_at	532.02	151.55	3.51	0.0380	
237112_at	449.03	79.36	3.51	0.0113	
230267_at	449.02	36.16	3.51	0.0005	na
225254_at	219.30	768.23	3.50	0.0296	MGC20255
231065_at	154.81	538.70	3.48	0.0455	PDE6D
218045_x_at	73.58	442.50	3.46	0.0257	PTMS
240069_at	119.30	442.05	3.45	0.0383	
232330_at	382.96	1320.92	3.45	0.0162	FLJ10803
222350_at	99.73	437.33	3.42	0.0377	
209600_s_at	435.38	46.70	3.40	0.0211	ACOX1
227193_at	632.74	186.36	3.40	0.0321	
229247_at	106.58	434.46	3.39	0.0281	FLJ37440
238476_at	431.80	92.22	3.37	0.0477	LOC153222
225711_at	930.97	276.12	3.37	0.0358	MGC33864
226743_at	516.11	153.07	3.37	0.0433	FLJ34922
1553905_at	97.31	428.48	3.35	0.0058	MGC34831
202331_at	273.60	914.15	3.34	0.0078	BCKDHA
238496_at	427.20	41.81	3.34	0.0088	WHSC1L1
229812_at	426.80	58.08	3.33	0.0358	USP31
221688_s_at	248.43	828.28	3.33	0.0405	C15orf12
211856_x_at	173.07	575.94	3.33	0.0160	CD28
219890_at	851.79	256.03	3.33	0.0374	CLECSF5
205100_at	238.10	791.62	3.32	0.0198	GFPT2
240971_x_at	61.11	423.34	3.31	0.0414	
204170_s_at	1063.48	322.49	3.30	0.0466	CKS2
204608_at	242.66	789.67	3.25	0.0330	ASL
227352_at	157.77	512.86	3.25	0.0200	FLJ35119
1562098_at	243.91	791.45	3.24	0.0265	
226152_at	415.27	52.48	3.24	0.0187	TTC7L1
230532_at	414.62	79.74	3.24	0.0236	MGC39350
205803_s_at	141.13	454.80	3.22	0.0306	TRPC1
1558147_a_at	437.79	1410.36	3.22	0.0100	na
229414_at	654.70	203.98	3.21	0.0037	PITPNC1
237309_at	171.98	550.63	3.20	0.0496	
1554544_a_at	407.62	74.16	3.18	0.0303	MBP
205807_s_at	335.94	1067.13	3.18	0.0195	TUFT1
213626_at	634.15	199.82	3.17	0.0402	FLJ14431
221800_s_at	54.85	405.90	3.17	0.0307	FLJ22175
1560250_s_at	154.05	488.22	3.17	0.0384	LOC284242
239096_at	414.39	1307.99	3.16	0.0466	

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
31835_at	301.62	949.95	3.15	0.0218	HRG
212373_at	780.45	248.02	3.15	0.0344	FEM1B
214828_s_at	58.20	402.21	3.14	0.0159	dJ222E13.2
219813_at	102.63	402.00	3.14	0.0172	LATS1
237337_at	197.47	616.78	3.12	0.0166	
228604_at	585.15	187.50	3.12	0.0122	MGC34648
203327_at	670.09	215.01	3.12	0.0060	IDE
229016_s_at	178.56	551.92	3.09	0.0010	TReP-132
207436_x_at	2064.25	6378.09	3.09	0.0457	
1556082_a_at	160.79	493.58	3.07	0.0179	
242294_at	57.28	391.98	3.06	0.0410	
1557193_at	243.13	737.28	3.03	0.0400	
221782_at	386.76	57.35	3.02	0.0357	ERdj5
1563572_at	44.81	386.25	3.02	0.0301	LOC152274
240326_at	2345.27	778.03	3.01	0.0357	
242914_at	258.41	778.23	3.01	0.0444	na
244021_at	138.94	416.98	3.00	0.0405	
223128_at	217.90	652.90	3.00	0.0041	H17
201382_at	110.78	383.17	2.99	0.0044	SIP
209704_at	382.41	38.90	2.99	0.0075	M96
202369_s_at	516.12	1540.60	2.98	0.0131	TRAM2
219002_at	1086.70	365.53	2.97	0.0439	FLJ21901
235759_at	690.70	233.22	2.96	0.0438	EFCBP1
1558078_at	42.28	378.29	2.96	0.0406	
235430_at	98.80	377.82	2.95	0.0324	C14orf43
1558612_a_at	176.14	518.91	2.95	0.0054	ATP1A4
1562673_at	82.22	377.08	2.95	0.0390	
207098_s_at	374.69	57.65	2.93	0.0194	MFN1
239370_at	64.58	373.76	2.92	0.0376	
244628_at	188.74	546.27	2.89	0.0119	
218394_at	173.73	501.29	2.89	0.0065	FLJ22386
215331_at	131.55	379.55	2.89	0.0318	KIAA1000
221771_s_at	368.96	57.70	2.88	0.0175	HSMPP8
226478_at	367.58	77.76	2.87	0.0335	TM7SF3
218799_at	43.32	366.43	2.86	0.0434	FLJ10349
232293_at	414.51	145.57	2.85	0.0116	HCAP-G
214867_at	420.79	1196.28	2.84	0.0246	NDST2
1558996_at	2501.35	881.42	2.84	0.0163	FOXP1
209088_s_at	482.78	1360.88	2.82	0.0120	UBN1
221594_at	123.10	360.40	2.82	0.0405	DKFZP564O0523
209840_s_at	2586.35	923.22	2.80	0.0366	LRRN3

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
226037_s_at	944.32	337.44	2.80	0.0473	
218692_at	222.64	622.12	2.79	0.0430	FLJ20366
1556035_s_at	1712.24	612.95	2.79	0.0406	ZNF207
1558969_a_at	332.38	923.98	2.78	0.0077	LOC132241
242502_at	21.62	353.25	2.76	0.0171	KCNH5
242407_at	342.47	944.18	2.76	0.0135	
1555522_s_at	1017.97	371.54	2.74	0.0101	CGI-27
1552552_s_at	123.13	349.01	2.73	0.0389	CLECSF11
230981_at	187.32	508.98	2.72	0.0133	CACRC
1563080_at	19.04	346.55	2.71	0.0217	
232289_at	167.64	452.29	2.70	0.0463	FLJ14167
214611_at	158.68	427.82	2.70	0.0371	GRIK1
240128_at	356.65	961.20	2.70	0.0169	
202692_s_at	132.76	357.06	2.69	0.0371	UBTF
213052_at	549.09	1476.63	2.69	0.0151	PRKAR2A
242607_at	962.80	359.16	2.68	0.0179	
1568954_s_at	343.11	58.23	2.68	0.0100	
203857_s_at	326.61	875.31	2.68	0.0045	PDIR
213370_s_at	510.70	1361.22	2.67	0.0364	SFMBT
244433_at	419.19	1116.88	2.66	0.0254	
221522_at	989.20	371.34	2.66	0.0024	DKFZP434L0718
1553132_a_at	338.53	33.62	2.64	0.0066	C14orf47
211220_s_at	67.78	337.55	2.64	0.0199	HSF2
205433_at	16.46	337.31	2.64	0.0401	BCHE
226212_s_at	152.80	401.02	2.62	0.0196	INSR
244147_at	59.09	335.55	2.62	0.0161	
223275_at	334.37	27.98	2.61	0.0218	PRMT6
227682_at	334.21	42.08	2.61	0.0429	
236610_at	2410.15	928.79	2.59	0.0155	
241826_x_at	27.06	332.13	2.59	0.0242	
213952_s_at	257.09	664.88	2.59	0.0305	ALOX5
243612_at	235.79	607.58	2.58	0.0082	NSD1
230847_at	384.37	988.28	2.57	0.0036	WRNIP1
209026_x_at	592.69	1515.31	2.56	0.0289	OK/SW-cl.56
244705_at	78.33	327.09	2.56	0.0056	
1561179_s_at	483.52	1231.08	2.55	0.0499	KIAA1950
203203_s_at	488.85	192.20	2.54	0.0069	HRB2
244207_at	382.02	966.89	2.53	0.0117	HARS2
213530_at	865.01	342.21	2.53	0.0374	RAB3GAP
212204_at	1921.67	761.83	2.52	0.0237	DKFZP564G2022
225882_at	77.08	322.82	2.52	0.0408	YEA

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
218659_at	743.98	1875.76	2.52	0.0027	KIAA1685
225974_at	322.01	25.56	2.52	0.0434	
202892_at	1165.40	464.12	2.51	0.0105	CDC23
1561038_at	46.99	321.03	2.51	0.0266	na
236322_at	2751.33	1099.21	2.50	0.0091	
219194_at	326.78	813.47	2.49	0.0351	SEMA4G
1557113_at	553.37	222.89	2.48	0.0426	LOC283588
203652_at	438.98	1087.22	2.48	0.0023	MAP3K11
1554599_x_at	314.13	769.95	2.45	0.0075	
242348_at	22.54	309.94	2.42	0.0135	FLJ25161
203357_s_at	309.54	37.50	2.42	0.0443	CAPN7
244328_x_at	6.56	307.87	2.41	0.0400	PAFAH1B1
234807_x_at	1058.72	2545.55	2.40	0.0275	
226864_at	307.29	37.03	2.40	0.0246	PKIA
1556339_a_at	345.63	829.52	2.40	0.0175	
201168_x_at	464.48	1114.34	2.40	0.0278	ARHGDIA
213022_s_at	212.14	507.23	2.39	0.0157	UTRN
231889_at	135.56	323.92	2.39	0.0155	RBAF600
244182_at	372.77	888.72	2.38	0.0119	na
205664_at	304.95	38.72	2.38	0.0231	KIN
202051_s_at	2300.27	971.49	2.37	0.0059	ZNF262
230346_x_at	408.25	966.63	2.37	0.0204	
204155_s_at	386.53	915.16	2.37	0.0273	KIAA0999
212828_at	357.06	151.11	2.36	0.0271	SYNJ2
1563796_s_at	234.24	547.37	2.34	0.0003	KIAA1970
203900_at	195.94	457.24	2.33	0.0283	KIAA0467
209556_at	228.14	531.95	2.33	0.0377	NCDN
1558163_at	297.82	36.77	2.33	0.0172	PEX13
229970_at	425.93	183.28	2.32	0.0026	DKFZP434E2318
1565674_at	1011.58	435.58	2.32	0.0076	FCGR2A
229488_at	316.29	734.48	2.32	0.0385	ZA20D1
222955_s_at	985.03	426.98	2.31	0.0179	HT011
226148_at	4796.79	2085.70	2.30	0.0223	HSPC063
1552899_at	331.57	762.27	2.30	0.0183	MGC34034
218998_at	1791.01	779.99	2.30	0.0275	FLJ20457
220315_at	210.55	480.80	2.28	0.0480	C12orf6
221238_at	123.74	291.65	2.28	0.0494	NSBP1
238596_at	241.80	550.17	2.28	0.0355	C10orf4
201748_s_at	273.10	621.31	2.28	0.0009	SAFB
204276_at	267.07	606.20	2.27	0.0059	na
204520_x_at	414.05	939.60	2.27	0.0484	BRD1

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
202471_s_at	399.72	906.63	2.27	0.0365	IDH3G
236742_at	651.15	1476.70	2.27	0.0039	
218895_at	249.81	564.12	2.26	0.0412	FLJ12455
221536_s_at	495.18	1117.35	2.26	0.0177	FLJ11301
226628_at	648.09	287.95	2.25	0.0020	THOC2
232096_x_at	578.95	1298.82	2.24	0.0466	
1554809_at	161.15	360.50	2.24	0.0128	na
222713_s_at	207.07	461.83	2.23	0.0236	FANCF
211977_at	362.46	808.29	2.23	0.0051	GPR107
227042_at	156.20	346.91	2.22	0.0147	LOC150223
230803_s_at	142.58	315.76	2.21	0.0411	DKFZP564B1162
217551_at	184.20	407.18	2.21	0.0492	na
214305_s_at	728.62	1604.89	2.20	0.0023	SF3B1
1564688_a_at	45.71	281.22	2.20	0.0350	FLJ40346
230120_s_at	145.58	319.37	2.19	0.0020	
217026_at	30.18	279.62	2.18	0.0030	
225708_at	256.92	557.44	2.17	0.0259	DKFZp434H247
1565662_at	535.79	1161.43	2.17	0.0047	MUC6
204020_at	2035.37	946.74	2.15	0.0105	PURA
208447_s_at	285.43	610.62	2.14	0.0259	PRPS1
225479_at	1456.69	682.26	2.14	0.0078	LOC116064
229021_at	2745.11	1294.55	2.12	0.0223	FLJ11175
1562792_at	395.78	835.82	2.11	0.0066	
240282_at	509.97	1075.97	2.11	0.0021	WDR1
201957_at	488.12	1028.02	2.11	0.0127	PPP1R12B
203572_s_at	386.77	814.37	2.11	0.0094	TAF6
241799_x_at	766.40	1613.65	2.11	0.0256	
1553528_a_at	268.67	48.39	2.10	0.0264	TAF5
236235_at	18.16	268.58	2.10	0.0037	ITCH
216036_x_at	532.17	1110.81	2.09	0.0337	KIAA1037
1555427_s_at	266.84	43.71	2.08	0.0409	NSAP1
1558233_s_at	266.17	28.69	2.08	0.0127	
207627_s_at	265.98	98.99	2.08	0.0173	TFCP2
201924_at	2646.48	1275.30	2.08	0.0088	MLLT2
228930_at	478.70	992.51	2.07	0.0341	LOC123722
212047_s_at	345.19	714.17	2.07	0.0021	DKFZP566H073
212625_at	487.19	1006.83	2.07	0.0173	STX10
231835_at	281.50	576.34	2.05	0.0106	MGC26818
225335_at	380.02	776.95	2.04	0.0491	
218962_s_at	261.48	54.62	2.04	0.0302	FLJ13576
230734_x_at	904.76	443.14	2.04	0.0163	STRN

Age Group	Newborn	Juvenile			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
229350_x_at	501.17	1020.59	2.04	0.0302	FLJ14464
211004_s_at	349.54	710.04	2.03	0.0060	ALDH3B1
235843_at	234.49	476.29	2.03	0.0481	
1558306_at	26.20	257.18	2.01	0.0367	na
202738_s_at	1321.84	659.15	2.01	0.0394	PHKB
240823_at	104.96	256.25	2.00	0.0161	
212427_at	364.35	182.56	2.00	0.0144	KIAA0368
Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
1405_i_at	68.55	2855.50	22.31	0.0093	CCL5
201108_s_at	85.26	1959.04	15.31	0.0478	THBS1
229822_at	56.68	1833.31	14.32	0.0192	PARVG
201904_s_at	342.61	4467.37	13.04	0.0480	C3orf8
201125_s_at	109.05	1286.44	10.05	0.0362	ITGB5
208791_at	77.40	1269.89	9.92	0.0211	CLU
233898_s_at	1281.39	130.93	9.79	0.0375	DKFZP564O1863
208159_x_at	252.93	2146.31	8.49	0.0046	DDX11
226119_at	3083.18	384.97	8.01	0.0249	LOC115294
212855_at	964.64	77.55	7.54	0.0320	KIAA0276
222483_at	365.14	2708.43	7.42	0.0382	MGC4342
212948_at	197.45	1327.42	6.72	0.0224	CAMTA2
1569206_at	2346.46	353.75	6.63	0.0210	MGC40368
203665_at	64.32	843.44	6.59	0.0161	HMOX1
217778_at	104.43	827.98	6.47	0.0206	SLC39A1
1555659_a_at	84.97	826.56	6.46	0.0452	TREML1
200766_at	58.65	818.61	6.40	0.0247	CTSD
213222_at	825.78	132.05	6.25	0.0349	PLCB1
222553_x_at	912.68	154.68	5.90	0.0463	OXR1
208581_x_at	58.25	754.92	5.90	0.0057	MT1X
226099_at	1032.71	181.77	5.68	0.0383	ELL2
209453_at	119.36	669.68	5.23	0.0302	SLC9A1
225290_at	668.59	86.87	5.22	0.0400	
212567_s_at	90.46	660.55	5.16	0.0065	MAP4
1554062_at	886.88	173.10	5.12	0.0370	XG
227224_at	1001.40	196.05	5.11	0.0011	FLJ10244
213262_at	759.19	149.30	5.08	0.0064	SACS
238781_at	1128.74	223.91	5.04	0.0204	SFRS12
202807_s_at	144.06	721.06	5.01	0.0285	TOM1
228198_s_at	93.75	640.49	5.00	0.0030	LOC150763
223243_s_at	775.99	155.46	4.99	0.0491	C1orf22

Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
203227_s_at	65.25	632.98	4.95	0.0396	SAS
230886_at	2163.80	447.38	4.84	0.0273	
213025_at	1310.94	271.71	4.82	0.0068	FLJ20274
202262_x_at	128.30	608.18	4.74	0.0428	DDAH2
244020_at	124.58	605.86	4.73	0.0280	
201613_s_at	78.39	602.19	4.70	0.0037	RUVBL1
211968_s_at	599.03	105.70	4.68	0.0229	HSPCA
225207_at	109.09	598.55	4.68	0.0033	PDK4
228381_at	2702.31	578.39	4.67	0.0294	ATF7IP2
205961_s_at	593.95	81.53	4.64	0.0341	PSIP2
218747_s_at	56.72	587.79	4.59	0.0132	TAPBP-R
224365_s_at	581.47	88.35	4.54	0.0309	TIGD7
227146_at	680.92	152.12	4.48	0.0225	SOXN
1558027_s_at	746.05	168.19	4.44	0.0295	PRKAB2
229798_s_at	317.41	1382.71	4.36	0.0255	BRI3
214626_s_at	139.22	605.26	4.35	0.0085	G2AN
203328_x_at	231.53	1000.55	4.32	0.0366	IDE
220507_s_at	54.07	548.44	4.28	0.0337	UPB1
217264_s_at	732.79	171.21	4.28	0.0430	
227685_at	547.10	88.43	4.27	0.0153	TMF1
1569573_at	541.90	44.03	4.23	0.0297	
212012_at	909.22	215.08	4.23	0.0117	D2S448
208893_s_at	537.01	42.25	4.20	0.0319	DUSP6
204655_at	676.60	2827.61	4.18	0.0427	CCL5
204291_at	1177.85	284.00	4.15	0.0325	KIAA0335
225295_at	911.10	224.43	4.06	0.0039	KIAA1265
203517_at	691.07	173.48	3.98	0.0386	MTX2
231426_at	508.82	111.44	3.98	0.0367	
227015_at	202.32	797.35	3.94	0.0270	LOC57168
201745_at	1046.62	266.51	3.93	0.0046	PTK9
219574_at	1215.54	312.52	3.89	0.0176	FLJ20668
229879_at	321.54	1231.59	3.83	0.0008	
202557_at	488.32	74.65	3.82	0.0094	STCH
222195_s_at	142.57	543.15	3.81	0.0488	NAP1
204993_at	383.38	1437.38	3.75	0.0076	GNAZ
229414_at	654.70	176.30	3.71	0.0119	PITPNC1
242625_at	1708.31	464.54	3.68	0.0113	cig5
241133_at	1007.29	3698.06	3.67	0.0480	
243286_at	1443.35	394.16	3.66	0.0449	
219009_at	115.95	467.14	3.65	0.0152	C14orf93
207735_at	85.33	466.93	3.65	0.0463	RNF125

Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
219396_s_at	176.88	629.03	3.56	0.0463	NEIL1
218599_at	85.33	453.94	3.55	0.0333	REC8
219399_at	448.25	24.21	3.50	0.0183	LIN7C
220305_at	111.10	446.95	3.49	0.0057	MGC3260
217854_s_at	310.76	1081.04	3.48	0.0029	POLR2E
226366_at	655.40	189.53	3.46	0.0207	SHPRH
201548_s_at	181.42	626.15	3.45	0.0300	PLU-1
235331_x_at	440.37	91.98	3.44	0.0222	MGC16202
228301_x_at	199.91	686.25	3.43	0.0403	NDUFB10
219002_at	1086.70	318.52	3.41	0.0060	FLJ21901
226780_s_at	105.34	436.33	3.41	0.0318	na
222103_at	2099.59	617.65	3.40	0.0322	ATF1
226437_at	91.68	430.55	3.36	0.0172	LOC90522
204735_at	300.31	1003.77	3.34	0.0264	PDE4A
226681_at	768.24	230.13	3.34	0.0302	UBE2H
213507_s_at	3343.29	11159.29	3.34	0.0466	KPNB1
238496_at	427.20	35.23	3.34	0.0039	WHSC1L1
205434_s_at	637.33	2125.99	3.34	0.0370	AAK1
215566_x_at	349.90	1166.91	3.33	0.0013	LYPLA2
204645_at	827.41	251.48	3.29	0.0359	CCNT2
232383_at	419.93	70.41	3.28	0.0097	TFEC
228961_at	419.76	107.38	3.28	0.0191	FLJ35954
204905_s_at	499.18	153.15	3.26	0.0410	TXNDC5
235385_at	1960.62	603.83	3.25	0.0444	FLJ20668
228087_at	584.24	180.64	3.23	0.0491	LOC90693
213808_at	477.68	149.16	3.20	0.0282	na
225144_at	999.83	312.73	3.20	0.0227	BMPR2
228095_at	454.48	142.34	3.19	0.0182	PHF14
206896_s_at	657.05	206.12	3.19	0.0411	FLJ00058
206873_at	407.72	69.55	3.19	0.0228	CA6
209434_s_at	406.97	81.65	3.18	0.0304	PPAT
213320_at	698.74	221.97	3.15	0.0465	PRMT3
222871_at	177.93	559.67	3.15	0.0185	FLJ10748
219534_x_at	47.01	400.63	3.13	0.0471	CDKN1C
240172_at	530.57	169.62	3.13	0.0324	LOC51290
222197_s_at	616.55	198.40	3.11	0.0465	
206583_at	607.57	195.64	3.11	0.0072	FLJ20344
204425_at	280.96	871.92	3.10	0.0388	ARHGAP4
244803_at	313.25	967.06	3.09	0.0048	
1553743_at	469.85	152.35	3.08	0.0100	LOC151194
226885_at	822.95	267.88	3.07	0.0113	

Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
206770_s_at	393.00	119.33	3.07	0.0170	SLC35A3
229371_at	1349.11	439.95	3.07	0.0379	
215690_x_at	472.68	1447.77	3.06	0.0330	GPAA1
228749_at	459.82	150.77	3.05	0.0493	KIAA1571
202906_s_at	620.21	203.70	3.04	0.0267	NBS1
213700_s_at	342.03	1040.11	3.04	0.0420	PKM2
234050_at	1228.41	404.99	3.03	0.0468	TAGAP
242136_x_at	1960.45	648.17	3.02	0.0151	ZRANB1
224448_s_at	157.09	473.78	3.02	0.0077	MGC14833
241027_at	384.18	72.50	3.00	0.0348	OPA1
233833_at	221.00	661.54	2.99	0.0243	
209704_at	382.41	30.65	2.99	0.0002	M96
203156_at	1584.66	531.63	2.98	0.0322	AKAP11
212819_at	89.66	381.17	2.98	0.0441	ASB1
226637_at	921.46	310.18	2.97	0.0123	UBE2H
217518_at	754.93	254.65	2.96	0.0418	FER1L3
236994_at	774.09	261.84	2.96	0.0304	na
224060_s_at	588.36	199.65	2.95	0.0028	CGI-30
222425_s_at	198.64	584.88	2.94	0.0366	PDIP38
203710_at	792.63	269.87	2.94	0.0282	ITPR1
218394_at	173.73	510.15	2.94	0.0329	FLJ22386
204038_s_at	169.48	495.04	2.92	0.0246	EDG2
213021_at	156.94	456.53	2.91	0.0280	GOSR1
229618_at	500.60	172.20	2.91	0.0217	SNX16
235630_at	178.47	518.03	2.90	0.0361	
225755_at	451.15	1308.29	2.90	0.0026	MGC35097
239647_at	93.00	370.97	2.90	0.0269	CHST13
230740_at	505.01	1453.61	2.88	0.0300	EHD3
202438_x_at	368.20	82.70	2.88	0.0438	na
228258_at	185.41	532.36	2.87	0.0350	
203290_at	109.26	366.78	2.87	0.0069	HLA-DQA1
229449_at	281.38	804.84	2.86	0.0381	
221957_at	650.20	1842.55	2.83	0.0417	PDK3
206958_s_at	1943.51	687.53	2.83	0.0231	UPF3A
202830_s_at	177.61	502.07	2.83	0.0480	G6PT1
218236_s_at	612.20	216.59	2.83	0.0096	PRKCN
213930_at	861.00	304.63	2.83	0.0218	na
226646_at	446.38	1251.54	2.80	0.0274	KLF2
203611_at	612.11	1713.93	2.80	0.0348	TERF2
210527_x_at	731.48	2047.72	2.80	0.0018	TUBA2
242294_at	57.28	358.24	2.80	0.0166	

Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
203035_s_at	208.81	582.79	2.79	0.0295	PIAS3
225775_at	255.98	714.39	2.79	0.0326	MGC50844
213372_at	507.85	182.00	2.79	0.0332	LOC152559
37796_at	283.65	791.44	2.79	0.0426	LRRN4
211460_at	429.50	154.61	2.78	0.0390	TTTY9
219736_at	354.78	98.85	2.77	0.0272	TRIM36
244778_x_at	583.79	1616.46	2.77	0.0485	
204140_at	236.72	652.52	2.76	0.0106	TPST1
226897_s_at	352.15	110.98	2.75	0.0330	HSPC055
231035_s_at	1139.11	414.46	2.75	0.0086	LOC220213
243213_at	1006.61	366.36	2.75	0.0213	STAT3
225239_at	542.73	1488.74	2.74	0.0030	
1552375_at	169.25	462.70	2.73	0.0042	ZNF333
223506_at	501.32	184.44	2.72	0.0359	LOC84524
1557430_at	345.38	66.50	2.70	0.0070	LOC147670
208896_at	825.24	306.45	2.69	0.0129	DDX18
201404_x_at	1353.81	505.33	2.68	0.0147	PSMB2
229891_x_at	522.24	195.02	2.68	0.0131	
235061_at	570.15	213.00	2.68	0.0477	DKFZp761G058
203655_at	161.15	431.12	2.68	0.0374	XRCC1
218951_s_at	419.05	1120.52	2.67	0.0053	FLJ11323
201139_s_at	712.60	266.93	2.67	0.0434	SSB
233480_at	368.39	979.69	2.66	0.0134	MGC3222
209175_at	806.01	303.23	2.66	0.0180	SEC23IP
225005_at	471.50	1252.93	2.66	0.0323	PHF13
204156_at	339.98	75.92	2.66	0.0025	KIAA0999
209891_at	22.61	339.47	2.65	0.0042	AD024
235607_at	401.69	151.76	2.65	0.0161	HNRPD
1553132_a_at	338.53	115.25	2.64	0.0410	C14orf47
222868_s_at	391.91	1035.98	2.64	0.0000	IL18BP
225455_at	1139.63	436.65	2.61	0.0178	STAF42
213269_at	448.11	172.11	2.60	0.0416	LOC57209
203459_s_at	564.10	1462.34	2.59	0.0447	VPS16
202169_s_at	1464.33	565.84	2.59	0.0037	AASDHPPT
215268_at	34.44	329.40	2.57	0.0165	KIAA0754
221664_s_at	464.89	1194.68	2.57	0.0018	F11R
202950_at	630.32	245.67	2.57	0.0364	CRYZ
202770_s_at	924.31	361.95	2.55	0.0456	CCNG2
1555274_a_at	544.29	213.29	2.55	0.0465	KIAA1724
1552927_at	435.33	171.73	2.53	0.0075	MGC45404
226852_at	429.81	169.60	2.53	0.0200	MTA3

Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
223207_x_at	304.80	770.09	2.53	0.0486	PHP14
212599_at	2968.74	1176.33	2.52	0.0075	AUTS2
235843_at	234.49	590.39	2.52	0.0357	
221536_s_at	495.18	1241.67	2.51	0.0077	FLJ11301
225134_at	292.63	733.22	2.51	0.0086	FLJ14800
227060_at	266.79	668.29	2.50	0.0260	TNFRSF19L
226943_at	54.39	319.59	2.50	0.0276	
225882_at	77.08	318.60	2.49	0.0361	YEA
226285_at	858.87	345.30	2.49	0.0015	M11S1
218929_at	442.45	178.05	2.49	0.0175	CARF
220140_s_at	694.63	1720.98	2.48	0.0209	SNX11
201416_at	1694.52	684.31	2.48	0.0206	SOX4
244512_at	30.76	316.54	2.47	0.0158	
235844_at	316.47	66.87	2.47	0.0353	PHTF1
222839_s_at	735.45	297.52	2.47	0.0466	PAPOLG
213737_x_at	4581.77	1865.67	2.46	0.0137	
238273_at	197.99	486.01	2.45	0.0486	
211930_at	827.28	337.16	2.45	0.0304	na
225350_s_at	2067.35	5071.32	2.45	0.0426	FLJ13456
220768_s_at	774.71	319.09	2.43	0.0115	CSNK1G3
222448_s_at	1433.75	593.36	2.42	0.0104	UMP-CMPK
201913_s_at	534.65	1285.41	2.40	0.0256	NBP
232486_at	811.99	1949.50	2.40	0.0478	KIAA1484
226864_at	307.29	31.68	2.40	0.0148	PKIA
225731_at	966.55	402.70	2.40	0.0330	KIAA1223
202654_x_at	801.38	334.23	2.40	0.0216	AXOT
226855_at	202.39	484.14	2.39	0.0218	
220239_at	277.27	654.09	2.36	0.0347	SBBI26
217456_x_at	3242.94	7648.60	2.36	0.0345	HLA-E
1569629_x_at	40.32	301.32	2.35	0.0497	na
238299_at	583.34	1365.29	2.34	0.0165	
200957_s_at	544.80	1273.61	2.34	0.0047	SSRP1
227375_at	335.03	143.45	2.34	0.0448	DKFZP566D1346
218331_s_at	733.90	314.34	2.33	0.0431	FLJ20360
206005_s_at	298.76	30.33	2.33	0.0004	C6orf84
217436_x_at	987.93	2285.28	2.31	0.0199	
1555978_s_at	737.13	319.09	2.31	0.0373	MRCL3
43544_at	297.32	686.55	2.31	0.0356	TRAP95
219276_x_at	494.36	214.27	2.31	0.0465	FLJ13657
208993_s_at	294.44	114.65	2.30	0.0133	
209284_s_at	1057.34	461.51	2.29	0.0277	RAP140

Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
205407_at	293.13	18.40	2.29	0.0046	RECK
228106_at	306.50	700.89	2.29	0.0007	FLJ20280
205986_at	736.02	1678.00	2.28	0.0252	AATK
213022_s_at	212.14	483.27	2.28	0.0053	UTRN
201165_s_at	1571.61	694.04	2.26	0.0470	PUM1
233946_at	346.74	783.46	2.26	0.0082	na
219118_at	94.83	288.85	2.26	0.0371	FKBP11
222731_at	2041.36	906.48	2.25	0.0290	ZDHHC2
214867_at	420.79	940.56	2.24	0.0119	NDST2
1558448_a_at	18.16	285.80	2.23	0.0077	
241438_at	285.68	82.32	2.23	0.0375	
1561144_at	108.34	285.55	2.23	0.0364	
209667_at	40.47	285.01	2.23	0.0250	CES2
242121_at	716.90	1594.55	2.22	0.0181	
201382_at	110.78	284.37	2.22	0.0029	SIP
214730_s_at	680.25	1510.71	2.22	0.0359	GLG1
241823_at	730.26	329.29	2.22	0.0277	
203607_at	190.59	422.00	2.21	0.0037	INPP5F
222756_s_at	372.18	822.51	2.21	0.0466	ARRB1
227042_at	156.20	345.07	2.21	0.0113	LOC150223
200035_at	448.67	990.81	2.21	0.0090	DULLARD
215764_x_at	560.19	1226.93	2.19	0.0334	AP2A2
200827_at	421.64	923.10	2.19	0.0150	PLOD
219630_at	381.08	833.12	2.19	0.0054	MAP17
238057_at	279.20	29.54	2.18	0.0462	
227962_at	544.22	1184.28	2.18	0.0261	ACOX1
200846_s_at	45.48	278.45	2.18	0.0466	PPP1CA
200621_at	586.74	1273.95	2.17	0.0415	CSRP1
1557966_x_at	968.73	446.48	2.17	0.0172	MGC61716
225431_x_at	1645.32	758.46	2.17	0.0262	ACY1L2
1553677_a_at	372.80	806.61	2.16	0.0383	MGC3794
227352_at	157.77	339.62	2.15	0.0354	FLJ35119
242073_at	635.89	297.38	2.14	0.0038	
231578_at	273.04	34.43	2.13	0.0098	GBP1
216233_at	156.65	334.09	2.13	0.0459	CD163
229129_at	768.33	360.65	2.13	0.0496	HNRPD
206555_s_at	967.62	457.42	2.12	0.0260	FLJ20274
219012_s_at	21.17	270.56	2.11	0.0010	C11ORF30
221575_at	328.15	692.76	2.11	0.0234	SCLY
228239_at	636.19	301.40	2.11	0.0303	C21orf51
239329_at	982.11	468.16	2.10	0.0061	RAB21

Age Group	Newborn	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
204521_at	611.45	292.05	2.09	0.0126	HSU79274
203572_s_at	386.77	807.47	2.09	0.0285	TAF6
217844_at	1146.23	2384.11	2.08	0.0159	NLIIF
1558233_s_at	266.17	40.71	2.08	0.0485	
242772_x_at	443.19	913.62	2.06	0.0015	
202330_s_at	524.85	1078.82	2.06	0.0418	UNG
204798_at	778.34	379.26	2.05	0.0050	MYB
219648_at	502.95	245.64	2.05	0.0284	FLJ10116
239603_x_at	1027.26	502.09	2.05	0.0063	
204069_at	355.56	726.82	2.04	0.0099	MEIS1
202874_s_at	1101.37	540.98	2.04	0.0206	ATP6V1C1
202795_x_at	855.42	1739.34	2.03	0.0264	HRIHFB2122
1559490_at	652.54	1326.62	2.03	0.0114	MGC4126
202531_at	967.08	1965.05	2.03	0.0259	IRF1
212306_at	824.02	405.92	2.03	0.0396	CLASP2
236922_at	470.19	232.22	2.02	0.0413	
213387_at	1257.39	624.39	2.01	0.0410	KIAA1240
226895_at	554.31	1112.65	2.01	0.0195	NFIC
204164_at	287.44	576.69	2.01	0.0298	SIPA1
206715_at	962.14	480.53	2.00	0.0401	TFEC
213052_at	549.09	1098.90	2.00	0.0372	PRKAR2A
Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
219607_s_at	146.29	3796.82	25.95	0.0424	MS4A4A
207072_at	242.43	5654.21	23.32	0.0492	IL18RAP
206390_x_at	749.72	16650.55	22.21	0.0446	PF4
205033_s_at	1351.98	29923.18	22.13	0.0162	DEFA1
202446_s_at	518.56	10132.74	19.54	0.0495	PLSCR1
225447_at	135.32	2604.11	19.24	0.0332	GPD2
235568_at	2452.87	40793.35	16.63	0.0478	LOC199675
205863_at	2180.02	35998.71	16.51	0.0353	S100A12
200934_at	115.50	2098.95	16.40	0.0478	DEK
229285_at	20.48	1985.36	15.51	0.0045	RNASEL
226277_at	128.89	1929.12	14.97	0.0465	COL4A3BP
238439_at	188.21	2632.62	13.99	0.0247	MGC22805
201694_s_at	801.86	10970.27	13.68	0.0432	EGR1
220496_at	454.82	5359.07	11.78	0.0383	CLEC2
224587_at	60.97	1461.32	11.42	0.0478	PC4
227038_at	129.40	1433.68	11.08	0.0252	MGC26963
201061_s_at	1145.01	12341.17	10.78	0.0439	STOM

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
227438_at	68.56	1366.58	10.68	0.0325	LAK
221505_at	60.89	1338.11	10.45	0.0425	ANP32E
225633_at	417.51	4363.10	10.45	0.0455	LOC147991
208708_x_at	149.53	1511.50	10.11	0.0447	EIF5
209301_at	232.90	2272.33	9.76	0.0314	CA2
202441_at	233.09	2153.67	9.24	0.0341	KEO4
203310_at	100.39	1141.73	8.92	0.0481	STXBP3
222692_s_at	102.88	1137.16	8.88	0.0354	FAD104
217986_s_at	89.84	1107.97	8.66	0.0336	BAZ1A
226280_at	132.41	1123.65	8.49	0.0050	BNIP2
218865_at	326.84	2741.00	8.39	0.0029	FLJ22390
206698_at	340.78	2786.41	8.18	0.0385	XK
209063_x_at	130.32	1053.65	8.09	0.0242	PAIP1
212688_at	455.84	3597.44	7.89	0.0472	PIK3CB
219035_s_at	283.34	2173.06	7.67	0.0458	RNF34
212157_at	1204.88	159.44	7.56	0.0368	SDC2
201111_at	176.51	1331.95	7.55	0.0339	CSE1L
1562597_at	925.40	115.08	7.23	0.0087	
229615_at	112.31	923.26	7.21	0.0174	
219697_at	1202.76	172.88	6.96	0.0268	HS3ST2
240131_at	122.85	879.33	6.87	0.0246	
202078_at	179.04	1217.50	6.80	0.0314	COPS3
209513_s_at	158.45	1073.73	6.78	0.0424	MGC10940
235973_at	857.88	120.33	6.70	0.0168	TRIP11
220034_at	148.72	991.14	6.66	0.0158	IRAK3
212631_at	279.73	1863.42	6.66	0.0437	na
210156_s_at	215.99	1435.51	6.65	0.0257	PCMT1
208933_s_at	286.45	1871.37	6.53	0.0324	FLJ10359
226910_at	137.77	891.68	6.47	0.0277	LOC51122
226105_at	137.63	889.55	6.46	0.0126	na
236081_at	43.33	817.14	6.38	0.0214	
1569401_at	150.31	953.96	6.35	0.0198	CLL-1
226338_at	96.24	797.75	6.23	0.0389	DKFZp762O076
201407_s_at	333.93	2075.36	6.21	0.0309	PPP1CB
221260_s_at	79.88	787.86	6.16	0.0369	C12orf22
202055_at	159.78	980.59	6.14	0.0447	KPNA1
213119_at	369.64	2260.16	6.11	0.0453	SLC36A1
203196_at	132.12	806.36	6.10	0.0375	ABCC4
203996_s_at	865.56	142.56	6.07	0.0156	C21orf2
231996_at	768.21	98.80	6.00	0.0045	N4BP2
208775_at	478.88	2859.05	5.97	0.0400	XPO1

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
237097_at	755.40	105.65	5.90	0.0308	SPTBN5
244185_at	1156.89	197.55	5.86	0.0137	METAP2
227368_at	125.71	746.57	5.83	0.0300	C6orf166
217167_x_at	74.76	738.02	5.77	0.0206	
225519_at	178.98	1024.27	5.72	0.0387	PPP4R2
202776_at	175.83	993.07	5.65	0.0179	HSU15552
215987_at	721.91	84.14	5.64	0.0335	PDZGEF1
238738_at	704.34	49.28	5.50	0.0261	PSMD7
202745_at	106.79	702.29	5.49	0.0026	USP8
229642_at	1271.04	232.16	5.47	0.0044	ARHGEF7
205368_at	695.61	55.93	5.43	0.0104	KIAA0773
234219_at	850.88	157.55	5.40	0.0343	
212250_at	295.32	1583.97	5.36	0.0209	LYRIC
227215_at	683.92	69.24	5.34	0.0073	
220180_at	681.91	103.59	5.33	0.0083	SE57-1
205689_at	717.63	134.94	5.32	0.0049	FLJ11383
225678_at	903.37	172.10	5.25	0.0301	RPC8
205098_at	1495.32	7835.56	5.24	0.0467	CCR1
208716_s_at	111.84	670.19	5.24	0.0023	LOC54499
203333_at	51.11	668.42	5.22	0.0010	KIFAP3
200749_at	82.51	665.92	5.20	0.0177	RAN
242338_at	1238.93	241.30	5.13	0.0355	
228791_at	705.36	137.41	5.13	0.0075	MGC61550
226152_at	52.48	654.51	5.11	0.0152	TTC7L1
242280_x_at	648.43	92.97	5.07	0.0020	CPEB4
217094_s_at	212.11	1071.96	5.05	0.0073	ITCH
224734_at	93.34	646.75	5.05	0.0027	HMGB1
204125_at	401.20	2020.36	5.04	0.0360	CIA30
224832_at	100.77	643.92	5.03	0.0045	DUSP16
205505_at	126.70	642.09	5.02	0.0110	GCNT1
242738_s_at	639.09	104.87	4.99	0.0260	ATBF1
210147_at	632.39	52.42	4.94	0.0133	ART3
1559950_at	630.73	51.41	4.93	0.0169	
1557540_at	629.17	109.14	4.92	0.0410	na
204212_at	652.90	134.95	4.84	0.0127	PTE1
207034_s_at	615.77	111.74	4.81	0.0392	GLI2
230261_at	157.79	756.36	4.79	0.0446	SIAT8D
226221_at	84.01	605.77	4.73	0.0206	KIAA1432
1567107_s_at	151.77	717.66	4.73	0.0285	TPM4
212779_at	142.10	665.55	4.68	0.0189	KIAA1109
219531_at	110.90	599.50	4.68	0.0453	FLJ10565

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
209666_s_at	212.00	986.93	4.66	0.0259	CHUK
233433_at	840.94	183.70	4.58	0.0183	
205839_s_at	585.95	127.89	4.58	0.0148	BZRAP1
242003_at	585.70	61.33	4.58	0.0274	
208975_s_at	130.57	593.62	4.55	0.0402	KPNB1
206197_at	602.12	133.13	4.52	0.0090	NME5
212536_at	692.08	3129.66	4.52	0.0424	ATP11B
212500_at	127.11	572.73	4.47	0.0186	FLJ14547
227987_at	85.47	572.52	4.47	0.0115	CHAC
203398_s_at	604.71	135.38	4.47	0.0029	GALNT3
216942_s_at	103.64	570.49	4.46	0.0176	CD58
1569947_at	568.87	115.74	4.44	0.0110	
209066_x_at	85.46	568.46	4.44	0.0172	UQCRB
243579_at	196.93	870.37	4.42	0.0319	MSI2
206007_at	563.81	43.80	4.40	0.0164	PRG4
208782_at	164.84	725.64	4.40	0.0188	FSTL1
242778_at	843.27	191.95	4.39	0.0471	LPXN
212007_at	203.75	891.79	4.38	0.0378	UBXD2
219620_x_at	203.71	887.05	4.35	0.0111	SLC34A3
204905_s_at	666.40	153.15	4.35	0.0138	TXNDC5
201614_s_at	83.09	555.51	4.34	0.0391	RUVBL1
1559654_s_at	874.37	201.65	4.34	0.0128	
204615_x_at	479.36	2071.68	4.32	0.0415	IDI1
229800_at	621.74	143.92	4.32	0.0369	
202545_at	71.83	550.83	4.30	0.0339	PRKCD
222572_at	80.03	549.60	4.29	0.0237	
202538_s_at	150.13	643.60	4.29	0.0270	DKFZP564O123
233943_x_at	547.60	115.57	4.28	0.0481	FAPP2
217851_s_at	88.83	545.47	4.26	0.0424	C20orf45
201821_s_at	86.30	544.29	4.25	0.0328	TIMM17A
244321_at	541.91	43.06	4.23	0.0260	FLJ12377
201513_at	111.20	536.86	4.19	0.0285	TSN
225611_at	112.03	534.31	4.17	0.0048	KIAA0303
217512_at	583.36	139.97	4.17	0.0452	KNG
204898_at	529.69	114.46	4.14	0.0072	SAP30
232647_at	531.43	128.59	4.13	0.0031	MGC39650
217264_s_at	706.97	171.21	4.13	0.0209	
219210_s_at	227.70	939.70	4.13	0.0332	LOC51762
1561089_at	628.85	152.54	4.12	0.0144	na
201298_s_at	1314.32	5403.27	4.11	0.0437	C2orf6
215613_at	821.16	200.59	4.09	0.0091	ADAM12

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
203725_at	466.35	1907.13	4.09	0.0301	GADD45A
204955_at	549.19	134.52	4.08	0.0473	SRPX
239544_at	1192.90	292.94	4.07	0.0168	SETBP1
240106_at	607.80	149.43	4.07	0.0174	MGC4170
229467_at	520.11	65.49	4.06	0.0452	PCBP2
226743_at	153.07	619.47	4.05	0.0055	FLJ34922
219496_at	515.64	58.68	4.03	0.0383	FLJ21870
230123_at	758.78	189.04	4.01	0.0183	FLJ10420
218904_s_at	223.61	894.70	4.00	0.0314	FLJ10110
240290_at	948.69	237.20	4.00	0.0448	
227721_at	985.16	247.59	3.98	0.0153	VIP
208849_at	508.66	92.33	3.97	0.0349	
203477_at	507.34	86.74	3.96	0.0418	COL15A1
244535_at	801.40	203.01	3.95	0.0084	
1570315_at	1054.81	269.50	3.91	0.0273	na
239247_at	1037.14	265.01	3.91	0.0158	
201001_s_at	374.97	1467.20	3.91	0.0125	UBE2V1
243676_at	1376.98	351.93	3.91	0.0454	C20orf59
1565863_at	500.29	55.11	3.91	0.0313	
222051_s_at	499.68	113.76	3.90	0.0437	E2F5
226161_at	41.47	497.69	3.89	0.0052	SLC30A6
228090_at	140.76	547.22	3.89	0.0046	NMNAT3
211367_s_at	52.39	496.64	3.88	0.0008	CASP1
219153_s_at	550.67	141.99	3.88	0.0270	FLJ13710
201234_at	371.18	1434.37	3.86	0.0196	ILK
220881_at	494.46	100.03	3.86	0.0243	LMO2
237337_at	616.78	159.77	3.86	0.0092	
230267_at	36.16	493.77	3.86	0.0016	na
219160_s_at	491.63	118.95	3.84	0.0006	PAPOLG
1566646_at	490.94	119.05	3.84	0.0391	LOC149086
212076_at	923.51	241.75	3.82	0.0120	MLL
224994_at	487.87	74.17	3.81	0.0406	CAMK2D
220178_at	834.44	219.10	3.81	0.0121	FZR1
205049_s_at	486.59	107.68	3.80	0.0036	CD79A
205260_s_at	107.38	486.49	3.80	0.0404	ACYP1
238614_x_at	531.62	139.94	3.80	0.0257	ZNF430
218979_at	88.66	485.65	3.79	0.0126	FLJ12888
1557480_a_at	909.02	239.83	3.79	0.0021	LOC284121
235215_at	485.07	95.52	3.79	0.0392	na
1562671_s_at	949.05	251.00	3.78	0.0010	
232386_at	482.78	29.23	3.77	0.0102	KIAA1421

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
243742_at	479.53	68.08	3.75	0.0114	
243855_at	477.12	70.83	3.73	0.0422	
210406_s_at	1263.85	4709.25	3.73	0.0382	RAB6A
242431_at	660.31	177.25	3.73	0.0288	
52741_at	923.71	248.58	3.72	0.0315	FLJ40452
1566001_at	1401.89	377.46	3.71	0.0122	
210858_x_at	917.83	248.28	3.70	0.0303	ATM
242177_at	713.88	193.40	3.69	0.0188	
240282_at	1075.97	292.02	3.68	0.0028	WDR1
209967_s_at	33.26	471.52	3.68	0.0072	CREM
244700_at	653.79	177.72	3.68	0.0487	SEC61B
203693_s_at	548.00	2009.03	3.67	0.0177	E2F3
211744_s_at	301.76	1105.06	3.66	0.0085	CD58
200688_at	950.58	259.90	3.66	0.0382	SF3B3
237461_at	465.43	116.13	3.64	0.0324	PYPAF3
211863_x_at	965.18	265.60	3.63	0.0245	HFE
240400_at	464.24	95.97	3.63	0.0377	
230860_at	106.94	463.50	3.62	0.0155	
242059_at	462.85	82.37	3.62	0.0281	
244627_at	813.49	225.31	3.61	0.0271	DKFZP586B1621
217534_at	1017.92	282.48	3.60	0.0192	PAK2
235662_at	780.55	216.76	3.60	0.0301	MGC15407
242407_at	944.18	262.71	3.59	0.0107	
242550_at	617.30	172.31	3.58	0.0237	
224341_x_at	526.92	1883.06	3.57	0.0452	TLR4
204332_s_at	525.19	1867.64	3.56	0.0157	AGA
200813_s_at	81.29	454.80	3.55	0.0218	PAFAH1B1
209512_at	58.44	453.63	3.54	0.0017	MGC10940
208943_s_at	412.77	1462.06	3.54	0.0385	TLOC1
218297_at	138.90	491.68	3.54	0.0242	FLJ13397
209137_s_at	414.37	1460.34	3.52	0.0134	USP10
233083_at	451.10	57.19	3.52	0.0467	FLJ13105
1562364_at	451.08	19.53	3.52	0.0188	na
216444_at	450.29	34.99	3.52	0.0198	
227949_at	448.46	105.29	3.50	0.0294	H17739
203321_s_at	1243.16	355.16	3.50	0.0141	KIAA0863
212610_at	584.02	2042.00	3.50	0.0270	PTPN11
233902_at	657.60	188.91	3.48	0.0312	GUCA1C
207262_at	444.25	44.24	3.47	0.0454	APOF
235619_at	443.00	115.34	3.46	0.0304	ASB4
224480_s_at	503.02	1739.24	3.46	0.0216	MGC11324

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
1569906_s_at	524.61	151.87	3.45	0.0420	C20orf104
201798_s_at	383.44	1322.62	3.45	0.0171	FER1L3
214668_at	1030.78	300.05	3.44	0.0217	C13orf1
1569573_at	436.66	44.03	3.41	0.0379	
235338_s_at	57.18	436.55	3.41	0.0162	SETDB2
217518_at	867.78	254.65	3.41	0.0347	FER1L3
1553693_s_at	613.13	180.33	3.40	0.0244	FLJ14431
221522_at	371.34	1261.38	3.40	0.0073	DKFZP434L0718
242011_at	1033.64	304.72	3.39	0.0230	na
1553561_at	506.16	149.30	3.39	0.0340	TAS2R50
205038_at	386.39	1305.21	3.38	0.0487	ZNFN1A1
1565661_x_at	605.20	179.56	3.37	0.0174	FUT6
218739_at	713.68	2404.69	3.37	0.0288	ABHD5
239371_at	444.56	132.12	3.36	0.0419	
224295_at	430.39	43.02	3.36	0.0281	
229086_at	747.24	223.57	3.34	0.0143	LOC148898
39835_at	427.56	45.06	3.34	0.0122	SBF1
225068_at	599.73	2002.76	3.34	0.0369	C3IP1
235086_at	95.11	427.12	3.34	0.0381	THBS1
202918_s_at	50.31	426.73	3.33	0.0312	PREI3
1562716_at	508.08	152.47	3.33	0.0417	LOC284632
231244_at	426.50	55.51	3.33	0.0350	CAS1
220509_at	425.02	24.34	3.32	0.0044	C13orf10
237719_x_at	424.81	17.11	3.32	0.0212	
210116_at	65.53	424.10	3.31	0.0078	SH2D1A
217315_s_at	643.09	194.15	3.31	0.0190	KLK13
237006_at	1416.74	4692.28	3.31	0.0404	
212365_at	969.61	292.81	3.31	0.0452	MYO1B
224679_at	111.49	423.19	3.31	0.0412	MESDC2
209460_at	443.42	1460.22	3.29	0.0231	NPD009
236621_at	742.88	226.09	3.29	0.0261	
218578_at	78.23	419.46	3.28	0.0472	C1orf28
235800_at	662.60	202.28	3.28	0.0253	
212036_s_at	73.65	419.05	3.27	0.0312	PNN
1555145_at	591.75	180.82	3.27	0.0414	LOC126731
238999_at	501.36	153.29	3.27	0.0454	
1559582_at	1917.36	586.85	3.27	0.0203	
1557778_at	467.30	143.21	3.26	0.0167	na
239306_at	416.01	116.95	3.25	0.0325	C14orf93
232293_at	145.57	470.78	3.23	0.0201	HCAP-G
236692_at	175.90	567.55	3.23	0.0349	na

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
207847_s_at	563.94	175.63	3.21	0.0453	MUC1
217627_at	1027.12	319.94	3.21	0.0447	FLJ30921
210102_at	214.27	685.70	3.20	0.0056	LOH11CR2A
211464_x_at	1213.01	379.55	3.20	0.0434	CASP6
215957_at	408.36	65.74	3.19	0.0494	UBE2D1
230466_s_at	631.85	2012.42	3.18	0.0184	
225411_at	55.76	406.68	3.18	0.0168	FLJ14681
221390_s_at	405.98	39.62	3.17	0.0261	MTMR8
233467_s_at	660.79	208.60	3.17	0.0324	PHEMX
228661_s_at	252.19	798.84	3.17	0.0216	
201416_at	2163.97	684.31	3.16	0.0269	SOX4
1562546_at	403.85	95.51	3.16	0.0128	
222564_at	403.10	65.44	3.15	0.0344	POGK
234362_s_at	621.78	197.48	3.15	0.0338	CTLA4
230852_at	464.84	147.68	3.15	0.0258	MGC2793
1557810_at	402.55	28.36	3.14	0.0448	
243134_at	401.78	62.28	3.14	0.0300	
1553536_at	1041.13	331.91	3.14	0.0252	MBNL2
1561749_at	1191.80	380.48	3.13	0.0152	
218970_s_at	227.84	712.31	3.13	0.0228	CGI-32
1555814_a_at	147.90	462.36	3.13	0.0190	ARHA
223087_at	219.41	685.45	3.12	0.0448	LOC55862
202394_s_at	610.90	196.08	3.12	0.0028	ABCF3
212997_s_at	302.44	941.95	3.11	0.0101	TLK2
1559093_at	858.83	276.94	3.10	0.0379	na
232432_s_at	127.01	396.56	3.10	0.0490	SLC30A5
233968_at	396.37	37.00	3.10	0.0250	CST11
212888_at	403.03	1241.88	3.08	0.0359	DICER1
239603_x_at	1547.04	502.09	3.08	0.0129	
212037_at	511.23	1565.31	3.06	0.0409	PNN
236167_at	391.30	57.11	3.06	0.0224	
224743_at	735.57	2244.13	3.05	0.0169	FLJ20421
221773_at	323.91	987.06	3.05	0.0025	ELK3
234757_at	771.47	253.26	3.05	0.0420	FLJ22624
207501_s_at	389.87	54.96	3.05	0.0106	FGF12
243511_at	703.22	231.02	3.04	0.0039	
1561311_at	389.51	35.13	3.04	0.0194	
228186_s_at	592.78	194.89	3.04	0.0402	THSD2
201595_s_at	355.80	1077.84	3.03	0.0486	LEREPO4
224514_x_at	615.47	203.30	3.03	0.0280	IL17RC
1561210_at	387.37	59.34	3.03	0.0424	

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
214023_x_at	386.68	77.52	3.02	0.0076	TUBB
210607_at	386.65	52.84	3.02	0.0139	FLT3LG
209471_s_at	148.31	447.97	3.02	0.0470	FNTA
1561187_at	633.17	209.70	3.02	0.0224	
204216_s_at	128.35	385.60	3.00	0.0361	FLJ11806
1559976_at	641.02	213.43	3.00	0.0212	
214377_s_at	481.74	160.40	3.00	0.0312	JAK1
223646_s_at	567.94	189.25	3.00	0.0498	CYorf15B
235935_at	424.26	141.53	3.00	0.0148	na
223894_s_at	271.84	813.79	2.99	0.0313	FTS
213986_s_at	663.67	222.01	2.99	0.0074	C19orf6
219194_at	813.47	272.54	2.98	0.0317	SEMA4G
216232_s_at	543.99	182.37	2.98	0.0217	GCN1L1
1570153_at	381.60	41.79	2.98	0.0079	FLJ20449
215245_x_at	298.37	886.83	2.97	0.0267	FMR1
213227_at	70.18	379.97	2.97	0.0088	PGRMC2
1564209_at	379.88	43.09	2.97	0.0354	LOC282980
236146_at	379.58	110.59	2.97	0.0462	NSAP1
229126_at	555.13	187.26	2.96	0.0476	FLJ10936
201098_at	590.80	1748.57	2.96	0.0378	COPB2
228204_at	33.49	378.78	2.96	0.0124	PSMB4
1552520_at	786.48	266.43	2.95	0.0222	FLJ30668
238718_at	377.55	30.35	2.95	0.0236	HRASLS3
239759_at	377.23	49.62	2.95	0.0197	
232574_at	377.12	91.70	2.95	0.0076	XYLT1
241499_at	376.56	88.52	2.94	0.0206	
236624_at	759.61	258.55	2.94	0.0109	
229172_at	716.62	243.95	2.94	0.0463	HSPA12B
229629_at	128.48	377.31	2.94	0.0312	
206127_at	798.25	271.83	2.94	0.0491	ELK3
230395_at	211.01	617.33	2.93	0.0488	
212783_at	873.82	298.92	2.92	0.0280	RBBP6
203327_at	215.01	625.82	2.91	0.0172	IDE
202611_s_at	207.08	602.06	2.91	0.0187	CRSP2
1553024_at	371.87	63.17	2.91	0.0172	G30
208741_at	131.73	382.08	2.90	0.0444	SAP18
242249_at	370.89	105.45	2.90	0.0303	
1555711_x_at	370.80	64.23	2.90	0.0330	
1561905_at	1304.25	450.50	2.90	0.0140	
1552649_a_at	370.53	55.92	2.89	0.0226	LOC117584
1558356_at	370.46	54.99	2.89	0.0400	UACA

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
206460_at	369.87	38.41	2.89	0.0218	SHREW1
208546_x_at	91.41	369.42	2.89	0.0076	HIST1H4G
225658_at	333.06	960.08	2.88	0.0279	LOC339745
209337_at	302.49	871.35	2.88	0.0167	PSIP2
225477_s_at	516.41	1485.53	2.88	0.0421	NR2C2
231806_s_at	603.27	209.73	2.88	0.0199	STK36
207367_at	762.39	265.07	2.88	0.0178	ATP12A
212351_at	494.73	172.05	2.88	0.0343	EIF2B5
210039_s_at	416.23	1194.02	2.87	0.0187	PRKCQ
213480_at	857.64	2460.23	2.87	0.0442	VAMP4
241857_at	555.98	194.77	2.85	0.0245	RAET1E
203989_x_at	365.07	110.64	2.85	0.0413	F2R
238145_at	987.12	348.32	2.83	0.0299	
206904_at	815.49	288.35	2.83	0.0394	MATN1
1560550_at	621.87	219.98	2.83	0.0442	na
216294_s_at	1026.90	363.35	2.83	0.0190	KIAA1109
218554_s_at	637.82	225.71	2.83	0.0128	ASH1L
240307_at	724.48	256.97	2.82	0.0146	
224271_x_at	360.78	76.46	2.82	0.0008	FKSG44
1569374_at	721.13	256.10	2.82	0.0487	
239021_at	360.02	63.82	2.81	0.0124	
218414_s_at	967.34	343.99	2.81	0.0125	NDE1
214363_s_at	2099.37	5885.19	2.80	0.0064	MATR3
204340_at	156.60	438.71	2.80	0.0417	CXorf12
233023_at	358.23	31.74	2.80	0.0075	
201663_s_at	139.56	390.29	2.80	0.0143	SMC4L1
244752_at	484.38	173.24	2.80	0.0487	LOC220929
203202_at	71.74	357.06	2.79	0.0002	HRB2
214163_at	409.49	146.84	2.79	0.0483	LOC51668
206415_at	356.46	21.40	2.78	0.0492	TLL1
224856_at	738.98	2057.00	2.78	0.0295	FKBP5
204495_s_at	1298.32	466.50	2.78	0.0068	DKFZP434H132
208523_x_at	489.27	176.15	2.78	0.0469	HIST1H2BI
217594_at	355.28	35.61	2.78	0.0191	KIAA0191
224216_at	354.86	38.10	2.77	0.0068	
58916_at	354.85	93.02	2.77	0.0014	MGC2376
220452_x_at	1539.85	555.73	2.77	0.0198	
209546_s_at	218.09	604.23	2.77	0.0130	APOL1
226829_at	416.22	150.78	2.76	0.0161	FLJ14564
201618_x_at	519.36	188.79	2.75	0.0479	GPAA1
230936_at	987.97	359.37	2.75	0.0325	TSARG3

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
238476_at	92.22	351.49	2.75	0.0462	LOC153222
213140_s_at	84.74	350.62	2.74	0.0117	SS18L1
226438_at	279.00	763.72	2.74	0.0066	SNTB1
243741_at	714.44	261.12	2.74	0.0425	
219905_at	655.77	1787.21	2.73	0.0320	ERMAP
202247_s_at	474.17	174.16	2.72	0.0438	MTA1
234463_at	348.16	100.33	2.72	0.0120	
239165_at	752.82	276.95	2.72	0.0314	
227273_at	347.91	124.96	2.72	0.0175	
228104_at	742.86	273.44	2.72	0.0347	PLXNA4
226851_at	327.03	888.36	2.72	0.0376	LYPLAL1
1563080_at	346.55	11.40	2.71	0.0057	
237174_at	438.76	162.43	2.70	0.0314	
231602_at	1374.92	509.69	2.70	0.0061	
218698_at	190.08	512.28	2.70	0.0301	MMRP19
208188_at	884.12	328.21	2.69	0.0314	KRT9
213374_x_at	442.86	1192.33	2.69	0.0267	HIBCH
1560851_at	344.48	44.92	2.69	0.0264	
240149_at	344.37	41.12	2.69	0.0157	FLJ22087
218041_x_at	594.94	1597.24	2.68	0.0299	SLC38A2
215136_s_at	280.15	750.19	2.68	0.0474	OIP2
205005_s_at	402.08	150.53	2.67	0.0312	NMT2
208315_x_at	842.44	315.54	2.67	0.0257	TRAF3
204831_at	173.06	461.12	2.66	0.0109	CDK8
203403_s_at	345.02	918.71	2.66	0.0174	RNF6
1556153_s_at	611.44	229.71	2.66	0.0315	MAIL
202301_s_at	793.22	298.68	2.66	0.0064	FLJ11021
216697_at	339.68	34.22	2.65	0.0187	TRIO
209412_at	46.67	339.23	2.65	0.0410	TMEM1
238164_at	355.43	937.88	2.64	0.0267	RNTRE
204650_s_at	886.19	335.99	2.64	0.0108	APBB3
233673_at	978.25	371.06	2.64	0.0158	LOC339524
208882_s_at	434.22	1138.81	2.62	0.0121	DD5
244828_x_at	467.10	178.65	2.61	0.0145	LOC92345
222627_at	541.41	1413.91	2.61	0.0392	HCC8
229247_at	434.46	166.45	2.61	0.0218	FLJ37440
230091_at	162.98	424.83	2.61	0.0091	SRPK2
214028_x_at	333.22	58.53	2.60	0.0357	TDRD3
207323_s_at	933.99	358.77	2.60	0.0343	MBP
231103_at	852.23	327.48	2.60	0.0301	
202892_at	464.12	1207.79	2.60	0.0084	CDC23

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
1564287_at	633.44	244.05	2.60	0.0334	LOC144776
218826_at	997.55	385.10	2.59	0.0191	HSNOV1
235902_at	721.43	278.59	2.59	0.0095	na
1556339_a_at	829.52	320.67	2.59	0.0068	
242967_at	329.62	88.25	2.58	0.0196	
234752_x_at	329.36	16.06	2.57	0.0051	DMD
239452_at	1675.60	651.57	2.57	0.0461	CCBP2
213018_at	171.98	441.19	2.57	0.0052	ODAG
225318_at	362.88	930.42	2.56	0.0469	KIAA0725
235331_x_at	327.89	91.98	2.56	0.0220	MGC16202
203746_s_at	379.73	970.79	2.56	0.0187	HCCS
225207_at	234.16	598.55	2.56	0.0271	PDK4
1562408_at	1086.38	425.11	2.56	0.0001	na
244705_at	327.09	33.14	2.56	0.0295	
203250_at	1164.35	2974.87	2.55	0.0375	RBM16
206706_at	782.12	306.51	2.55	0.0355	NTF3
230241_at	46.49	325.11	2.54	0.0145	
214657_s_at	451.11	1145.17	2.54	0.0127	TncRNA
230098_at	332.83	842.63	2.53	0.0294	FLJ21615
203618_at	771.00	304.77	2.53	0.0257	FAIM2
225958_at	966.89	382.59	2.53	0.0145	M6PR
233560_x_at	701.15	278.46	2.52	0.0256	MCM8
223707_at	455.70	181.12	2.52	0.0472	ST5
208868_s_at	142.17	357.20	2.51	0.0143	GABARAPL1
1562468_at	798.11	2002.53	2.51	0.0210	
231224_x_at	954.42	382.15	2.50	0.0179	
204181_s_at	873.60	350.34	2.49	0.0466	ZNF297B
238823_at	1216.07	488.97	2.49	0.0271	WBP3
219747_at	478.02	192.25	2.49	0.0475	FLJ23191
225088_at	482.29	194.06	2.49	0.0113	FLJ31153
236882_at	317.81	25.72	2.48	0.0073	
207627_s_at	98.99	317.34	2.48	0.0144	TFCP2
244784_at	316.19	16.14	2.47	0.0093	DHX57
205471_s_at	70.98	316.05	2.47	0.0076	DACH
223189_x_at	1034.60	419.68	2.47	0.0104	MLL5
223958_s_at	315.02	74.89	2.46	0.0427	MGC12435
230624_at	314.99	126.06	2.46	0.0136	UCP4
222792_s_at	1234.42	501.97	2.46	0.0250	HSPC128
225853_at	1128.12	460.11	2.45	0.0492	GNPNAT1
1554537_at	1013.13	413.24	2.45	0.0216	HT007
226452_at	620.03	1518.55	2.45	0.0045	PDK1

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
1566037_at	682.12	279.59	2.44	0.0322	DGCR7
1568954_s_at	58.23	312.24	2.44	0.0048	
241342_at	149.73	364.83	2.44	0.0083	LOC157378
219236_at	1084.54	445.34	2.44	0.0014	FLJ22672
226628_at	287.95	700.51	2.43	0.0049	THOC2
222815_at	77.18	310.84	2.43	0.0401	
233161_at	1389.32	572.27	2.43	0.0123	
233132_at	616.95	255.44	2.42	0.0183	LOC96610
225086_at	34.94	308.00	2.41	0.0024	FLJ38426
230029_x_at	43.82	307.85	2.41	0.0063	KIAA2024
244628_at	546.27	227.15	2.40	0.0263	
231255_at	1456.24	606.01	2.40	0.0177	RHOIP3
216961_s_at	307.22	105.98	2.40	0.0006	
214305_s_at	1604.89	669.24	2.40	0.0143	SF3B1
205481_at	594.96	248.76	2.39	0.0326	ADORA1
223479_s_at	383.16	160.70	2.38	0.0482	C2orf9
238768_at	523.10	219.72	2.38	0.0456	
1558822_at	945.33	397.60	2.38	0.0393	
226917_s_at	389.20	921.55	2.37	0.0412	ANAPC4
219676_at	328.15	775.98	2.36	0.0181	ZNF435
224450_s_at	77.76	302.21	2.36	0.0292	RIOK1
1556555_at	727.82	308.64	2.36	0.0396	LOC285778
219991_at	1477.24	626.67	2.36	0.0257	SLC2A9
229891_x_at	459.40	195.02	2.36	0.0448	
211685_s_at	683.16	290.10	2.35	0.0409	NCALD
1556053_at	2165.74	920.13	2.35	0.0460	DNAJC7
202684_s_at	300.93	112.89	2.35	0.0304	RNMT
212238_at	1260.71	537.44	2.35	0.0231	ASXL1
204371_s_at	619.60	264.30	2.34	0.0132	KHSRP
1562698_x_at	2204.85	942.47	2.34	0.0290	LOC339988
223217_s_at	1346.18	576.22	2.34	0.0396	MAIL
229506_at	99.95	298.97	2.34	0.0258	
224712_x_at	837.84	358.79	2.34	0.0377	
227540_at	966.68	414.42	2.33	0.0237	SELB
226736_at	598.71	1395.31	2.33	0.0301	C14orf52
211095_at	403.76	173.94	2.32	0.0150	NF1
1552632_a_at	349.53	150.75	2.32	0.0354	KIAA1001
201518_at	640.81	1482.84	2.31	0.0396	CBX1
234347_s_at	599.03	259.25	2.31	0.0469	DENR
220742_s_at	1052.84	2424.92	2.30	0.0489	NGLY1
200764_s_at	317.57	731.23	2.30	0.0402	CTNNA1

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
211113_s_at	989.62	430.20	2.30	0.0391	ABCG1
224286_at	992.16	431.99	2.30	0.0373	C1QTNF5
204781_s_at	563.24	1292.49	2.29	0.0248	TNFRSF6
202166_s_at	564.74	1295.19	2.29	0.0137	PPP1R2
206229_x_at	583.92	254.99	2.29	0.0333	PAX2
232008_s_at	852.01	372.48	2.29	0.0250	BBX
228822_s_at	289.89	660.69	2.28	0.0348	USP16
219983_at	143.15	326.24	2.28	0.0419	HRASLS
240773_at	290.09	90.28	2.27	0.0279	
216573_at	290.08	39.96	2.27	0.0114	
209600_s_at	46.70	289.00	2.26	0.0199	ACOX1
202331_at	914.15	404.93	2.26	0.0043	BCKDHA
204983_s_at	67.19	288.16	2.25	0.0393	GPC4
211702_s_at	626.68	1405.60	2.24	0.0207	USP32
244182_at	888.72	397.48	2.24	0.0419	na
222989_s_at	476.01	1063.07	2.23	0.0030	UBQLN1
1555437_at	662.25	296.74	2.23	0.0394	KIAA1970
229697_at	637.39	285.75	2.23	0.0080	HIRIP3
206267_s_at	407.20	182.64	2.23	0.0377	MATK
201687_s_at	327.14	729.34	2.23	0.0279	API5
227236_at	589.67	1312.73	2.23	0.0450	TSPAN-2
224303_x_at	331.12	736.61	2.22	0.0064	NIN
232253_at	905.29	406.97	2.22	0.0158	
219700_at	607.38	273.08	2.22	0.0491	TEM7
1557319_at	1603.55	722.03	2.22	0.0199	
1555306_a_at	1103.77	497.36	2.22	0.0138	ECE2
208914_at	1547.21	697.51	2.22	0.0365	GGA2
207821_s_at	597.10	269.53	2.22	0.0016	PTK2
201627_s_at	108.93	281.61	2.20	0.0367	INSIG1
232786_at	281.55	72.06	2.20	0.0060	COG6
209357_at	39.09	281.44	2.20	0.0431	CITED2
219917_at	548.37	249.48	2.20	0.0443	ZCCHC4
213168_at	39.77	281.20	2.20	0.0063	SP3
47773_at	1336.71	608.59	2.20	0.0477	KIAA1332
221588_x_at	1253.69	571.00	2.20	0.0211	ALDH6A1
230888_at	931.88	425.41	2.19	0.0247	na
220050_at	913.84	417.65	2.19	0.0173	C9orf9
204481_at	606.24	277.77	2.18	0.0044	BRPF1
206414_s_at	399.64	872.03	2.18	0.0357	DDEF2
227179_at	325.24	707.58	2.18	0.0310	STAU2
59644_at	428.44	930.94	2.17	0.0105	BMP2K

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
204304_s_at	381.49	175.62	2.17	0.0442	PROM1
64438_at	923.40	425.58	2.17	0.0456	FLJ22222
235798_at	48.15	277.46	2.17	0.0371	
209585_s_at	69.70	276.69	2.16	0.0270	MINPP1
202637_s_at	1332.96	618.55	2.15	0.0036	ICAM1
37004_at	403.36	187.39	2.15	0.0391	SFTPB
217266_at	1344.38	625.68	2.15	0.0006	
233750_s_at	58.20	274.18	2.14	0.0446	C1orf25
219734_at	1276.13	596.31	2.14	0.0174	FLJ20174
210313_at	1214.58	568.19	2.14	0.0102	LIR9
241795_at	822.06	384.70	2.14	0.0119	
1563321_s_at	273.50	96.19	2.14	0.0245	MLL
202778_s_at	635.73	1357.86	2.14	0.0238	ZNF198
225269_s_at	1069.68	2284.25	2.14	0.0122	HCC-4
229773_at	737.57	1571.41	2.13	0.0012	SNAP23
222480_at	94.56	272.27	2.13	0.0385	UBE2Q
217207_s_at	1519.99	716.25	2.12	0.0093	BTNL3
1555464_at	557.33	262.77	2.12	0.0158	MDA5
216979_at	893.19	421.78	2.12	0.0296	NR4A3
218878_s_at	323.06	683.34	2.12	0.0126	SIRT1
241799_x_at	1613.65	763.39	2.11	0.0096	
224200_s_at	288.47	136.59	2.11	0.0390	RAD18
227775_at	677.44	321.16	2.11	0.0369	BRUNOL6
223213_s_at	333.94	704.07	2.11	0.0391	ZHX1
1555380_at	918.25	436.30	2.10	0.0388	ADAMTS4
223177_at	961.77	2022.77	2.10	0.0238	NT5C2L1
236961_at	989.46	471.27	2.10	0.0080	
209430_at	501.93	1050.66	2.09	0.0325	BTAF1
212450_at	437.38	914.80	2.09	0.0489	KIAA0256
1557768_at	925.80	442.84	2.09	0.0343	
244704_at	267.57	81.67	2.09	0.0190	NFYB
214283_at	1051.96	503.37	2.09	0.0115	LOC90410
201626_at	616.40	1284.39	2.08	0.0260	INSIG1
229362_at	266.41	53.73	2.08	0.0325	FLJ32312
1556464_a_at	1326.16	638.93	2.08	0.0176	
212427_at	182.56	377.45	2.07	0.0106	KIAA0368
235872_at	1399.47	677.18	2.07	0.0190	TERF2
219429_at	490.44	237.42	2.07	0.0373	FAXDC1
212568_s_at	70.09	264.24	2.06	0.0390	DLAT
1559094_at	231.05	476.04	2.06	0.0176	FBXO9
212301_at	412.14	846.55	2.05	0.0011	KIAA0252

Age Group	Juvenile	Elderly			
Probe Set ID	Ctrl Group Signal	Exp Group Signal	Absolute Adjusted Fold Change	P-Value	Gene Symbol
227394_at	1257.53	612.84	2.05	0.0164	na
206186_at	1302.84	635.35	2.05	0.0406	MPP3
218808_at	777.49	379.41	2.05	0.0227	FLJ10496
217945_at	523.02	1071.11	2.05	0.0435	BTBD1
236994_at	69.02	261.84	2.05	0.0090	na
1553523_at	573.01	280.12	2.05	0.0077	NALP14
31835_at	949.95	464.66	2.04	0.0130	HRG
225243_s_at	348.35	711.02	2.04	0.0430	SLMAP
242361_at	1041.43	510.93	2.04	0.0303	IMMT
218799_at	366.43	180.13	2.03	0.0433	FLJ10349
214241_at	1029.84	506.37	2.03	0.0125	NDUFB8
215786_at	861.00	425.07	2.03	0.0484	
211693_at	1106.80	547.12	2.02	0.0228	MGC27165
242604_at	258.40	32.85	2.02	0.0493	
1555522_s_at	371.54	749.58	2.02	0.0170	CGI-27
210070_s_at	803.59	399.88	2.01	0.0071	CPT1B
243852_at	446.79	222.36	2.01	0.0465	LUC7L2
209082_s_at	772.05	385.18	2.00	0.0456	COL18A1
202630_at	355.18	711.22	2.00	0.0232	APPBP2
241775_at	1155.40	2309.56	2.00	0.0091	C14orf163

APPENDIX D: CANDIDATE GENE DATABASE

Table 25: Candidate Gene Database.

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
ABL1	Homo sapiens v-abl Abelson murine leukemia viral oncogene homolog 1, transcript variant b	Oncogene	Elderly	NM_007313
ACD	Homo sapiens adrenocortical dysplasia homolog (mouse), transcript variant 2	Disease	Elderly	NM_022914
ACTA2	Homo sapiens cDNA FLJ36021 fis, clone TESTI2016568	Affymetrix	Juvenile	AK093340
ACTN3	Homo sapiens actinin, alpha 3	Transcription & Gene Regulation	Elderly	NM_001104
ADAM12	AU145357 HEMBA1 Homo sapiens cDNA clone HEMBA1004611 3-	Affymetrix	Juvenile	AU145357
ADAM12a	AU145357 HEMBA1 Homo sapiens cDNA clone HEMBA1004611 3-	Affymetrix	Juvenile	AU145357
AFP	Homo sapiens alpha-fetoprotein	Fetal Protein	Newborn	NM_001134
AGGF1	Homo sapiens angiogenic factor with G patch and FHA domains 1	Affymetrix	Elderly	NM_018046
AIF1	Homo sapiens allograft inflammatory factor 1, transcript variant 3	Immunology & Interferons	Elderly	NM_001623
AKT1	Homo sapiens v-akt murine thymoma viral oncogene homolog 1, transcript variant 1	Oncogene	Elderly	NM_005163
AMID	Homo sapiens apoptosis-inducing factor, mitochondrion-associated, 2 (AIFM2)	Mitochondria	Elderly	NM_032797
ANKH	Homo sapiens ankylosis, progressive homolog (mouse)	Bone	Elderly	NM_054027
APEX1	Homo sapiens APEX nuclease (multifunctional DNA repair enzyme) 1, transcript variant 1	DNA Damage & Growth Arrest	Elderly	NM_001641

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
APOE (1)	Human apolipoprotein E mRNA, complete cds	Fetal Protein	Newborn	M12529
APOE (2)	Homo sapiens apolipoprotein E	Fetal Protein	Newborn	NM_000041
ARMC7	Homo sapiens armadillo repeat containing 7	Affymetrix	Elderly	NM_024585
Art3a	Human putative mono-ADP-ribosyltransferase (htMART)	Affymetrix	Juvenile	U47054
Art3b	Human putative mono-ADP-ribosyltransferase (htMART)	Affymetrix	Juvenile	U47054
ASL	Homo sapiens argininosuccinate lyase, transcript variant 2	Affymetrix	Juvenile	NM_000048
ATF7IP2	DCB Homo sapiens cDNA clone DCBBOG12 5'	Affymetrix	Newborn	AV7169647
ATPAF2	Homo sapiens ATP synthase mitochondrial F1 complex assembly factor 2, nuclear gene encoding mitochondrial protein	Affymetrix	Elderly	NM_145691
AUF1 hnRNPD p37	Homo sapiens heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa) (HNRPD), transcript variant 4	Fetal Protein Isofrom	Newborn	NM_001003810
AUF1 hnRNPD p45	Homo sapiens heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa) (HNRPD), transcript variant 1	Adult Isoform	Adult	NM_031370
BAX-(all)	Homo sapiens BCL2-associated X protein, transcript variant epsilon	Apoptosis	Elderly	NM_138764
BAX-a/d	Homo sapiens BCL2-associated X protein, transcript variant alpha	Apoptosis	Elderly	NM_138761

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
BAX-all(e)	Homo sapiens BCL2-associated X protein, transcript variant epsilon	Apoptosis	Elderly	NM_138764
BAX-b	Homo sapiens BCL2-associated X protein, transcript variant beta	Apoptosis	Elderly	NM_004324
BAX-d	Homo sapiens BCL2-associated X protein, transcript variant delta	Apoptosis	Elderly	NM_138763
BAX-e	Homo sapiens BCL2-associated X protein, transcript variant epsilon	Apoptosis	Elderly	NM_138764
BAX-s	Homo sapiens BCL2-associated X protein, transcript variant sigma	Apoptosis	Elderly	NM_138765
BCKDHA	Homo sapiens branched chain keto acid dehydrogenase E1, alpha polypeptide	Affymetrix	Juvenile	NM_000709
BCL2A1	Homo sapiens BCL2-related protein A1	Apoptosis	Elderly	NM_004049
BGLAP	Homo sapiens bone gamma-carboxyglutamate (gla) protein (osteocalcin)	Bone	Elderly	NM_199173
BIRC5	Homo sapiens baculoviral IAP repeat-containing 5 (survivin), transcript variant 3	Apoptosis	Elderly	NM_001012271
c5229134	Homo sapiens, clone IMAGE:5229134	Affymetrix	Juvenile	BC037976
c5286506	Homo sapiens cDNA clone IMAGE:5286506	Affymetrix	Juvenile	BC043160
CABP7	Homo sapiens calcium binding protein 7	Affymetrix	Juvenile	NM_182527
CABYR	Homo sapiens calcium binding tyrosine-(Y)-phosphorylation regulated (fibrousheathin 2), transcript variant 1	Hormone	Juvenile	NM_012189

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
CAMK2D	Homo sapiens calcium/calmodulin-dependent protein kinase (CaM kinase) II delta, transcript variant 1	Growth Factor	Elderly	NM_172127
CASP2	Homo sapiens caspase 2, apoptosis-related cysteine peptidase (neural precursor cell expressed, developmentally down-regulated 2), transcript variant 1	Apoptosis	Elderly	NM_032982
CBL	Homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence	Oncogene	Elderly	NM_005188
CCL5	Homo sapiens chemokine (C-C motif) ligand 5	Affymetrix	Elderly	NM_002985
CCM2	Homo sapiens cerebral cavernous malformation 2, transcript variant 1	Disease	Elderly	NM_001029835
CCND1	Homo sapiens cyclin D1	Cyclin	Elderly	NM_053056
CD200	Homo sapiens CD200 molecule, transcript variant 2	Immunology & Interferons	Juvenile	NM_001004196
CD28	Homo sapiens CD28 molecule	Affymetrix	Juvenile	NM_006139
CD28	Homo sapiens CD28 molecule	Immunology & Interferons	Elderly	NM_006139
CD86	Homo sapiens CD86 molecule, transcript variant 1	Immunology & Interferons	Elderly	NM_175862
CDC2	Homo sapiens cell division cycle 2, G1 to S and G2 to M, transcript variant 1	Cyclin	Elderly	NM_001786
CDC25C	Homo sapiens cell division cycle 25 homolog C (S. pombe), transcript variant 2	Cyclin	Elderly	NM_022809
CDKN1A	Homo sapiens cyclin-dependent kinase inhibitor 1A (p21, Cip1), transcript variant 2	Cyclin	Elderly	NM_078467

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
CDKN1B	Homo sapiens cyclin-dependent kinase inhibitor 1B (p27, Kip1)	Cyclin	Elderly	NM_004064
CDKN2C	Homo sapiens cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4), transcript variant 1	Cyclin	Elderly	NM_001262
CFIX	Homo sapiens coagulation factor IX (plasma thromboplastic component, Christmas disease, hemophilia B) (F9)	Growth Factor	Juvenile	NM_000133
CGI-96	Novel human gene mapping to chomosome 22	Affymetrix	Juvenile	AL157851
CHR1orf28	zw89h01.r1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:784177 5- similar to contains Alu repetitive element.	Affymetrix	Newborn	AA447464
CIITA	Homo sapiens class II, major histocompatibility complex, transactivator (CIITA)	Transcription & Gene Regulation	Elderly	NM_000246
CLEC2	Homo sapiens C-type lectin domain family 1, member B (CLEC1B)	Affymetrix	Elderly	NM_016509
CLEC2a	Homo sapiens C-type lectin domain family 1, member B (CLEC1B)	Affymetrix	Elderly	NM_016509
CLEC2b	Homo sapiens C-type lectin domain family 1, member B (CLEC1B)	Affymetrix	Elderly	NM_016509
COL1A1 CTx	Homo sapiens collagen, type I, alpha 1	Bone	Elderly	NM_000088
COL1A2	Homo sapiens collagen, type I, alpha 2	Bone	Newborn	NM_000089
COL6A1a	Homo sapiens, alpha-1 (VI)	Affymetrix	Juvenile	M20776
COL6A1b	Homo sapiens, alpha-1 (VI)	Affymetrix	Juvenile	M20776

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
CTBP1	Homo sapiens C-terminal binding protein 1, transcript variant 2	Transcription & Gene Regulation	Elderly	NM_001012614
CTSB	Homo sapiens cathepsin B, transcript variant 2	Bone	Elderly	NM_147780
CTSK	Homo sapiens cathepsin K (pycnodysostosis)	Bone	Elderly	NM_000396
CTSL	Homo sapiens cathepsin L1 (CTSL1), transcript variant 1	Bone	Elderly	NM_001912
CXorf22	Homo sapiens chromosome X open reading frame 22 (CXorf22)	Affymetrix	Juvenile	NM_152632
CYP17A1	Homo sapiens cytochrome P450, family 17, subfamily A, polypeptide 1	Hormone	Juvenile	NM_000102
CYP1B1	Homo sapiens cytochrome P450, family 1, subfamily B, polypeptide 1	Hormone	Juvenile	NM_000104
CYP7B1	Homo sapiens cytochrome P450, family 7, subfamily B, polypeptide 1 (CYP7B1),	Hormone	Juvenile	NM_004820
CYTBC2	Homo sapiens mRNA for cytochrome b large subunit of complex II, complete cds	Affymetrix	Elderly	D49737
DDB2	Homo sapiens damage-specific DNA binding protein 2, 48kDa	DNA Damage & Growth Arrest	Elderly	NM_000107
DHEA	Homo sapiens sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1 (SULT2A1)	Hormone	Juvenile	NM_003167
DNCL2A-1	Homo sapiens dynein, light chain, roadblock-type 1 (DYNLRB1), transcript variant 1	Affymetrix	Juvenile	NM_014183
DNCL2A-2	Homo sapiens dynein, cytoplasmic, light polypeptide 2A, transcript variant 2	Affymetrix	Juvenile	NM_177953

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
DNCL2A-3	Homo sapiens dynein, cytoplasmic, light polypeptide 2A, transcript variant 3	Affymetrix	Juvenile	NM_177954
DNPEP	Homo sapiens aspartyl aminopeptidase	Affymetrix	Juvenile	NM_012100
DUSP6	Homo sapiens dual specificity phosphatase 6, mRNA (cDNA clone MGC:12852 IMAGE:3954486), complete cds	Affymetrix	Newborn	BC005047
DYRK2	Homo sapiens dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2, transcript variant 2	Growth Factor	Elderly	NM_006482
E2F1	Homo sapiens E2F transcription factor 1	Transcription & Gene Regulation	Elderly	NM_005225
E2IG2	Homo sapiens coiled-coil-helix-coiled-coil-helix domain containing 8	Hormone	Juvenile	NM_016565
ECGF1	Homo sapiens endothelial cell growth factor 1 (platelet-derived)	Growth Factor	Elderly	NM_001953
ELAVL1 HuR	Homo sapiens ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	Transcription & Gene Regulation	Elderly	NM_001419
EMD	Homo sapiens emerin (Emery-Dreifuss muscular dystrophy)	Disease	Elderly	NM_000117
ERBP	Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2)	Hormone	Juvenile	NM_014597
EREG	Homo sapiens epiregulin	Growth Factor	Elderly	NM_001432
ERF	Homo sapiens Ets2 repressor factor	Tumor Suppressor Genes	Elderly	NM_006494
ESR1	Homo sapiens estrogen receptor 1	Hormone	Juvenile	NM_000125

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
ESR2	Homo sapiens estrogen receptor 2 (ER beta), transcript variant a	Hormone	Juvenile	NM_001437
FACL6	AV727634 HTC Homo sapiens cDNA clone HTCAYH08 5-	Affymetrix	Newborn	AV727634
FKBP11	Homo sapiens FK506 binding protein 11, 19 kDa	Affymetrix	Juvenile	NM_016594
FKLF	Homo sapiens kruppel-like fetal and embryonic globin gene activator, complete cds	Fetal Protein	Newborn	AF272830
FLJ11078	Homo sapiens kelch-like 26 (Drosophila) (KLHL26)	Affymetrix	Juvenile	NM_018316
FLJ20245	Homo sapiens chromosome 9 open reading frame 167 (C9orf167)	Affymetrix	Elderly	NM_017723
FLJ20344a	Homo sapiens zinc finger protein 673 (ZNF673)	Affymetrix	Newborn	NM_017776
FLJ20344b	Homo sapiens zinc finger protein 673 (ZNF673)	Affymetrix	Newborn	NM_017776
FLJ20421	602136866F1 NIH_MGC_83 Homo sapiens cDNA clone IMAGE:4273120 5-	Affymetrix	Elderly	BF674724
FLJ21901	Homo sapiens FAST kinase domains 1 (FASTKD1)	Affymetrix	Newborn	NM_024622
FLJ22175	Homo sapiens chromosome 17 open reading frame 70 (C17orf70)	Affymetrix	Juvenile	NM_025161
FLJ22672 PAQR6	Homo sapiens progestin and adipoQ receptor family member VI, transcript variant 1	Affymetrix	Juvenile	NM_024897
FLJ30658	hn54d06.x1 NCI_CGAP_Co17 Homo sapiens cDNA clone IMAGE:3027467 3-	Affymetrix	Newborn	AW770868

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
FLJ35119	Homo sapiens chromosome 19 open reading frame 39 (C19orf39)	Affymetrix	Juvenile	NM_175871
FLJ35954	yi35b09.s1 Soares placenta Nb2HP Homo sapiens cDNA clone IMAGE:141209 3-	Affymetrix	Newborn	R66534
FLJ35982	Homo sapiens cDNA FLJ35982 fis, clone TESTI2013604	Affymetrix	Juvenile	AK093301
FLJ35982a	Homo sapiens cDNA FLJ35982 fis, clone TESTI2013604	Affymetrix	Juvenile	AK093301
FLJ35984	Homo sapiens cDNA FLJ35984 fis, clone TESTI2014097, highly similar to V_segment translation product	Affymetrix	Elderly	AK093303
FLJ37440	Homo sapiens hypothetical protein FLJ37440	Affymetrix	Juvenile	NM_153214
FLJ38628	Homo sapiens ring finger protein 185 (RNF185)	Affymetrix	Elderly	NM_152267
FLJ38745	Homo sapiens cDNA FLJ38745 fis, clone KIDNE2012291	Affymetrix	Juvenile	AK096064
FLJ43159	wr63b05.x1 NCI_CGAP_Ut1 Homo sapiens cDNA clone IMAGE:2492337 3-	Affymetrix	Juvenile	AI972146
GADD45A	Homo sapiens growth arrest and DNA-damage-inducible, transcript variant alpha	DNA Damage & Growth Arrest	Elderly	NM_001924
GADD45B	Homo sapiens growth arrest and DNA-damage-inducible, transcript variant beta	DNA Damage & Growth Arrest	Elderly	NM_015675
GAL	Homo sapiens galanin	Hormone	Juvenile	NM_015973
GFPT2	Homo sapiens glutamine-fructose-6-phosphate transaminase 2	Affymetrix	Juvenile	NM_005110

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
GGT1	Homo sapiens gamma-glutamyltransferase 1, transcript variant 1	Affymetrix	Juvenile	NM_005265
GHRH	Homo sapiens growth hormone releasing hormone	Hormone	Juvenile	NM_021081
GLO1	Homo sapiens glyoxalase I	Immunology & Interferons	Juvenile	NM_006708
GNAS	Homo sapiens GNAS complex locus, transcript variant 4	Housekeeping Gene	HSK	NM_016592
GNAS2	Homo sapiens GNAS complex locus, transcript variant 4	Housekeeping Gene	HSK	NM_016592
GNRH1	Homo sapiens gonadotropin-releasing hormone 1 (luteinizing-releasing hormone), transcript variant 1	Hormone	Juvenile	NM_000825
GNRH2	Homo sapiens gonadotropin-releasing hormone 2, transcript variant 1	Hormone	Juvenile	NM_001501
GNRHR	Homo sapiens gonadotropin-releasing hormone receptor, transcript variant 1	Hormone	Juvenile	NM_000406
GPR54	Homo sapiens KISS1 receptor (KISS1R)	Hormone	Juvenile	NM_032551
GRIN1 NR1-1	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 1, transcript variant NR1-1	Hormone	Juvenile	NM_000832
GRIN1 NR1-2	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 1, transcript variant NR1-2	Hormone	Juvenile	NM_021569
GRIN1 NR1-3	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 1, transcript variant NR1-3	Hormone	Juvenile	NM_007327
GRIN2A	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 2A	Hormone	Juvenile	NM_000833

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
GRIN2B	Homo sapiens glutamate receptor, ionotropic, N-methyl D-aspartate 2B	Hormone	Juvenile	NM_000834
GSTP1	Homo sapiens glutathione S-transferase pi	Affymetrix	Elderly	NM_000852
H17	Homo sapiens FAD-dependent oxidoreductase domain containing 1 (FOXRED1)	Affymetrix	Juvenile	NM_017547
HBA1	Homo sapiens hemoglobin, alpha 1	Hemoglobin	Adult	NM_000558
HBA2	Homo sapiens hemoglobin, alpha 2	Hemoglobin	Adult	NM_000517
HBB	Homo sapiens hemoglobin, beta	Hemoglobin	Adult	NM_000518
HBD	Homo sapiens hemoglobin, delta	Hemoglobin	Adult	NM_000519
HBE1	Homo sapiens hemoglobin, epsilon 1	Hemoglobin	Newborn	NM_005330
HBG1	Homo sapiens hemoglobin, gamma A	Hemoglobin	Newborn	NM_000559
HBG1n1	Novel transcript isoform of hemoglobin, gamma A	Hemoglobin	Newborn	
HBG1n2	Novel transcript isoform of hemoglobin, gamma A	Hemoglobin	Newborn	
HBG2	Homo sapiens hemoglobin, gamma G	Hemoglobin	Newborn	NM_000184
HBG2n2	Novel transcript isoform of hemoglobin, gamma G	Hemoglobin	Newborn	
HBG2n3	Novel transcript isoform of hemoglobin, gamma G	Hemoglobin	Newborn	

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
HBZ	Homo sapiens hemoglobin, zeta	Hemoglobin	Newborn	NM_005332
HBQ	Homo sapiens hemoglobin, theta 1	Hemoglobin	Adult	NM_005331
HIC2	Homo sapiens hypermethylated in cancer 2	Affymetrix	Elderly	NM_015094
HIF1A	Homo sapiens hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor), transcript variant 1	Transcription & Gene Regulation	Elderly	NM_001530
HOMER3	Homo sapiens homer homolog 3 (Drosophila)	Affymetrix	Juvenile	NM_004838
HPCAL4	Homo sapiens hippocalcin like 4	Affymetrix	Elderly	NM_016257
HRAS	Homo sapiens v-Ha-ras Harvey rat sarcoma viral oncogene homolog, transcript variant 2	Oncogene	Elderly	NM_176795
HRG	Homo sapiens histidine-rich glycoprotein	Affymetrix	Juvenile	NM_000412
HTATIP	Homo sapiens HIV-1 Tat interacting protein, 60kDa, transcript variant 1	DNA Damage & Growth Arrest	Elderly	NM_182710
HTR1E	Homo sapiens 5-hydroxytryptamine (serotonin) receptor 1E	Affymetrix	Juvenile	NM_000865
HTR7	Homo sapiens 5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled), transcript variant a	Affymetrix	Juvenile	NM_000872
IFNG	Homo sapiens interferon, gamma	Immunology & Interferons	Elderly	NM_000619
IGF1	Homo sapiens insulin-like growth factor 1 (somatomedin C)	Growth Factor	Elderly	NM_000618

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
IGF2	Homo sapiens insulin-like growth factor 2 (somatomedin A), transcript variant 1	Growth Factor	Elderly	NM_000612
IGFBP3	Homo sapiens insulin-like growth factor binding protein 3, transcript variant 1	Growth Factor	Elderly	NM_001013398
IGFBP5	Homo sapiens insulin-like growth factor binding protein 5	Growth Factor	Elderly	NM_000599
IL1A	Homo sapiens interleukin 1, alpha	Immunology & Interferons	Elderly	NM_000575
INHA	Homo sapiens inhibin, alpha	Bone	Juvenile	NM_002191
IRF1	Homo sapiens interferon regulatory factor 1	Immunology & Interferons	Elderly	NM_002198
ITIH4	Homo sapiens inter-alpha (globulin) inhibitor H4 (plasma Kallikrein-sensitive glycoprotein)	Affymetrix	Juvenile	NM_002218
ITSN2	Homo sapiens intersectin 2, transcript variant 1	Affymetrix	Newborn	NM_006277
KIAA0276	Homo sapiens mRNA for KIAA0276 gene, partial cds	Affymetrix	Newborn	D87466
KIAA0894	Homo sapiens KIAA0894 protein	Affymetrix	Juvenile	NM_014896
KIAA1265	Homo sapiens mRNA for KIAA1265 protein, partial cds	Affymetrix	Newborn	AB033091
KIAA2022	Homo sapiens mRNA for KIAA2022 protein	Affymetrix	Juvenile	AB095942
KISS-1	Homo sapiens KiSS-1 metastasis-suppressor	Hormone	Juvenile	NM_002256

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
KITLG	Homo sapiens KIT ligand, transcript variant b	Fetal Protein	Newborn	NM_000899
KL	Homo sapiens klotho, transcript variant 2	Bone	Elderly	NM_153683
KLF13	Homo sapiens Kruppel-like factor 13	Transcription & Gene Regulation	Elderly	NM_015995
LASS5	Homo sapiens LAG1 homolog, ceramide synthase 5 (S. cerevisiae)	Affymetrix	Elderly	NM_147190
LATS1	Homo sapiens LATS, large tumor suppressor, homolog 1 (Drosophila)	Affymetrix	Juvenile	NM_004690
LEP	Homo sapiens leptin (obesity homolog, mouse)	Hormone	Juvenile	NM_000230
LHB	Homo sapiens luteinizing hormone beta polypeptide	Hormone	Juvenile	NM_000894
LHCGR	Homo sapiens luteinizing hormone/choriogonadotropin receptor	Hormone	Juvenile	NM_000233
LMNA (norm)	Homo sapiens lamin A/C, transcript variant 1	Transcription & Gene Regulation	Elderly	NM_170707
LMNA (RT)	Homo sapiens lamin A/C, transcript variant 1	Transcription & Gene Regulation	Elderly	NM_170707
LMNA (spec)	Homo sapiens lamin A/C, transcript variant 1	Transcription & Gene Regulation	Elderly	NM_170707
LOC151194	Homo sapiens family with sequence similarity 119, member A (FAM119A)	Affymetrix	Newborn	NM_145280
LOC152274	Homo sapiens cDNA FLJ31836 fis, clone NT2RP7000041	Affymetrix	Juvenile	AK056398

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
LOC284242	Homo sapiens, clone IMAGE:5745916	Affymetrix	Juvenile	BC035844
LOH11CR2A	Homo sapiens loss of heterozygosity, 11, chromosomal region 2, gene A, mRNA (cDNA clone MGC:4904 IMAGE:3461486), complete cds	Affymetrix	Elderly	BC001234
LZTFL1	Homo sapiens leucine zipper transcription factor-like 1	Affymetrix	Newborn	NM_020347
MAD1L1	Homo sapiens MAD1 mitotic arrest deficient-like 1 (yeast), transcript variant 1	Cyclin	Elderly	NM_003550
MCPH1	Homo sapiens microcephaly, primary autosomal recessive 1	Tumor Suppressor Genes	Elderly	NM_024596
MDM2	Homo sapiens Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse), transcript variant MDM2	Tumor Suppressor Genes	Elderly	NM_002392
MEPE	Homo sapiens matrix, extracellular phosphoglycoprotein with ASARM motif (bone)	Bone	Elderly	NM_020203
MET	Homo sapiens met proto-oncogene (hepatocyte growth factor receptor)	Oncogene	Elderly	NM_000245
MGC14288	Homo sapiens chromosome 12 open reading frame 62 (C12orf62)	Affymetrix	Elderly	NM_032901
MGC20460	Homo sapiens proline rich 8 (PRR8)	Affymetrix	Juvenile	NM_053043
MGC39650	Homo sapiens mRNA; cDNA DKFZp434F0919 (from clone DKFZp434F0919)	Affymetrix	Juvenile	AL137531
MIF	Homo sapiens macrophage migration inhibitory factor (MIF) gene, complete cds	Fetal Protein	Newborn	L19686
MLL	Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	Disease	Elderly	NM_005933

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
MMP-13	Homo sapiens matrix metallopeptidase 13 (collagenase 3)	Bone	Elderly	NM_002427
MMP-14	Homo sapiens matrix metallopeptidase 14 (membrane-inserted)	Bone	Elderly	NM_004995
MMP-9	Homo sapiens matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	Bone	Elderly	NM_004994
MS4A4A	Homo sapiens membrane-spanning 4-domains, subfamily A, member 4, transcript variant 1	Affymetrix	Elderly	NM_024021
MS4A4Aa	Homo sapiens membrane-spanning 4-domains, subfamily A, member 4, transcript variant 1	Affymetrix	Elderly	NM_024021
MS4A4Ab	Homo sapiens membrane-spanning 4-domains, subfamily A, member 4, transcript variant 1	Affymetrix	Elderly	NM_024021
MT1X	Homo sapiens metallothionein 1X	Affymetrix	Elderly	NM_005952
MYC	Homo sapiens v-myc myelocytomatosis viral oncogene homolog (avian)	Oncogene	Elderly	NM_002467
NALP14	Homo sapiens NLR family, pyrin domain containing 14 (NLRP14)	Affymetrix	Juvenile	NM_176822
NBN	Homo sapiens nibrin, transcript variant 2	Disease	Elderly	NM_001024688
NDE1	Homo sapiens nudE nuclear distribution gene E homolog 1 (A. nidulans)	Affymetrix	Juvenile	NM_017668
NMI	Homo sapiens N-myc (and STAT) interactor	Oncogene	Elderly	NM_004688
NPPB	Homo sapiens natriuretic peptide precursor B	Bone	Elderly	NM_002521

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
NRAS	Homo sapiens neuroblastoma RAS viral (v-ras) oncogene homolog	Oncogene	Elderly	NM_002524
NTS	Homo sapiens neurotensin	Hormone	Juvenile	NM_006183
OGG1	Homo sapiens 8-oxoguanine DNA glycosylase (OGG1), nuclear gene encoding mitochondrial protein, transcript variant 1b	Mitochondria	Elderly	NM_016819
OPG TNFRSF11B	Homo sapiens tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	Bone	Elderly	NM_002546
OPGL RANKL TNFSF11	Homo sapiens tumor necrosis factor (ligand) superfamily, member 11, transcript variant 1	Bone	Elderly	NM_003701
OSGEP	Homo sapiens O-sialoglycoprotein endopeptidase	Affymetrix	Juvenile	NM_017807
OSM	Homo sapiens oncostatin M	Growth Factor	Elderly	NM_020530
OXTR	Homo sapiens oxytocin receptor	Hormone	Juvenile	NM_000916
PAQR6	Homo sapiens progestin and adipoQ receptor family member VI, transcript variant 1	Affymetrix	Juvenile	NM_024897
PDCD1	Homo sapiens programmed cell death 1	Programmed Cell Death	Elderly	NM_005018
PDCD10 CCM3	Homo sapiens programmed cell death 10, transcript variant 1	Programmed Cell Death	Elderly	NM_007217
PDCD11	Homo sapiens programmed cell death 11	Programmed Cell Death	Elderly	NM_014976
PDCD1LG2	Homo sapiens programmed cell death 1 ligand 2	Programmed Cell Death	Elderly	NM_025239

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
PDCD2L	Homo sapiens programmed cell death 2-like	Programmed Cell Death	Elderly	NM_032346
PDCD4	Homo sapiens programmed cell death 4 (neoplastic transformation inhibitor), transcript variant 2	Programmed Cell Death	Elderly	NM_145341
PDCD5	Homo sapiens programmed cell death 5	Programmed Cell Death	Elderly	NM_004708
PDCD6	Homo sapiens programmed cell death 6	Programmed Cell Death	Elderly	NM_013232
PDCD6IP	Homo sapiens programmed cell death 6 interacting protein	Programmed Cell Death	Elderly	NM_013374
PDCD7	Homo sapiens programmed cell death 7	Programmed Cell Death	Elderly	NM_005707
PDE6D	Homo sapiens phosphodiesterase 6D, cGMP-specific, rod, delta	Affymetrix	Juvenile	NM_002601
PGR	Homo sapiens progesterone receptor	Hormone	Juvenile	NM_000926
PIK3CA	Homo sapiens phosphoinositide-3-kinase, catalytic, alpha polypeptide	Oncogene	Elderly	NM_006218
PITPNC1	wc05c10.x1 NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2314290 3-	Affymetrix	Newborn	AI676095
PLEKHA8	Homo sapiens pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 8	Affymetrix	Juvenile	NM_032639
POLA1	Homo sapiens polymerase (DNA directed), alpha 1	Polymerase	Elderly	NM_016937
POLA2	Homo sapiens polymerase (DNA directed), alpha 2 (70kD subunit)	Polymerase	Elderly	NM_002689

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
POLB	Homo sapiens polymerase (DNA directed), beta	Polymerase	Elderly	NM_002690
POLD1	Homo sapiens polymerase (DNA directed), delta 1, catalytic subunit 125kDa	Polymerase	Elderly	NM_002691
POLE1	Homo sapiens polymerase (DNA directed), epsilon	Polymerase	Elderly	NM_006231
POLE2	Homo sapiens polymerase (DNA directed), epsilon 2 (p59 subunit)	Polymerase	Elderly	NM_002692
POLE3	Homo sapiens polymerase (DNA directed), epsilon 3 (p17 subunit)	Polymerase	Elderly	NM_017443
POLG	Homo sapiens polymerase (DNA directed), gamma	Polymerase	Elderly	NM_002693
POLH	Homo sapiens polymerase (DNA directed), eta	Polymerase	Elderly	NM_006502
POLI	Homo sapiens polymerase (DNA directed) iota	Polymerase	Elderly	NM_007195
POLK	Homo sapiens polymerase (DNA directed) kappa	Polymerase	Elderly	NM_016218
POLM	Homo sapiens polymerase (DNA directed), mu	Polymerase	Elderly	NM_013284
POLN	Homo sapiens polymerase (DNA directed) nu	Polymerase	Elderly	NM_181808
POLQ	Homo sapiens polymerase (DNA directed), theta	Polymerase	Elderly	NM_199420
POLR3F	Homo sapiens polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa	Polymerase	Elderly	NM_006466

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
POLR3K	Homo sapiens polymerase (RNA) III (DNA directed) polypeptide K, 12.3 kDa	Polymerase	Elderly	NM_016310
POLS	Homo sapiens polymerase (DNA directed) sigma	Polymerase	Elderly	NM_006999
POMC	Homo sapiens proopiomelanocortin (adrenocorticotropin/ beta-lipotropin/ alpha-melanocyte stimulating hormone/ beta-melanocyte stimulating hormone/ beta-endorphin), transcript variant 2	Hormone	Juvenile	NM_000939
POT1	Homo sapiens POT1 protection of telomeres 1 homolog (S. pombe), transcript variant 2	Telomeres	Elderly	NR_003102
PPARD	Homo sapiens peroxisome proliferator-activated receptor delta	Bone	Elderly	NM_006238
PPAT	Homo sapiens glutamine PRPP amidotransferase (GPAT)	Affymetrix	Newborn	U00238
PPOX	Homo sapiens protoporphyrinogen oxidase, nuclear gene encoding mitochondrial protein	Mitochondria	Juvenile	NM_000309
PRDX5	Homo sapiens peroxiredoxin 5, nuclear gene encoding mitochondrial protein, transcript variant 1	Mitochondria	Elderly	NM_012094
PRKCA	Homo sapiens protein kinase C, alpha	Cyclin	Elderly	NM_002737
PRL	Homo sapiens prolactin	Hormone	Juvenile	NM_000948
PTGER4	Homo sapiens prostaglandin E receptor 4 (subtype EP4)	Bone	Elderly	NM_000958
PTH	Homo sapiens parathyroid hormone	Hormone	Juvenile	NM_000315

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
PTMS	Homo sapiens parathymosin	Affymetrix	Juvenile	NM_002824
PTPN18	Homo sapiens protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	Affymetrix	Elderly	NM_014369
RAD50	Homo sapiens RAD50 homolog (S. cerevisiae), transcript variant 1	DNA Damage & Growth Arrest	Elderly	NM_005732
RAF1	Homo sapiens v-raf-1 murine leukemia viral oncogene homolog 1	Oncogene	Elderly	NM_002880
RaI	wq65b01.x1 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGE:2476105 3-	Affymetrix	Newborn	AW003297
RaIGPS2	Homo sapiens Ral GEF with PH domain and SH3 binding motif 2, transcript variant 1	Affymetrix	Newborn	NM_018037
RANK	Homo sapiens receptor activator of nuclear factor-kappa B	Growth Factor	Elderly	AF018253
RAPA-2 TRERF1	Homo sapiens mRNA for rapa-2 (rapa gene), transcript variant 3	Hormone	Juvenile	AJ277276
RARA	Homo sapiens retinoic acid receptor, alpha, transcript variant 1	Affymetrix	Juvenile	NM_000964
RB1	Homo sapiens retinoblastoma 1 (including osteosarcoma)	Tumor Suppressor Genes	Elderly	NM_000321
RBL1	Homo sapiens retinoblastoma-like 1 (p107), transcript variant 1	Tumor Suppressor Genes	Elderly	NM_002895
RBL2	Homo sapiens retinoblastoma-like 2 (p130)	Tumor Suppressor Genes	Elderly	NM_005611
REA	Homo sapiens prohibitin 2 (PHB2)	Hormone	Juvenile	NM_007273

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
RELA	Homo sapiens v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)	Oncogene	Elderly	NM_021975
RUNX2	Homo sapiens runt-related transcription factor 2, transcript variant 3	Transcription & Gene Regulation	Elderly	NM_004348
S15	Homo sapiens ribosomal protein S15 (RPS15)	Housekeeping Gene	HSK	NM_001018
SEMA4A	Homo sapiens sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	Affymetrix	Elderly	NM_022367
SH3GL1	Homo sapiens SH3-domain GRB2-like 1	Affymetrix	Juvenile	NM_003025
SHBG	Homo sapiens sex hormone-binding globulin	Hormone	Juvenile	NM_001040
SLC20A1	Homo sapiens solute carrier family 20 (phosphate transporter), member 1	Immunology & Interferons	Elderly	NM_005415
SLC39A4	Homo sapiens solute carrier family 39 (zinc transporter), member 4, transcript variant 1	Affymetrix	Juvenile	NM_017767
SMG5	Homo sapiens Smg-5 homolog, nonsense mediated mRNA decay factor (C. elegans)	Telomeres	Elderly	NM_015327
SMG6	Homo sapiens Smg-6 homolog, nonsense mediated mRNA decay factor (C. elegans)	Telomeres	Elderly	NM_017575
SMG7	Homo sapiens Smg-7 homolog, nonsense mediated mRNA decay factor (C. elegans), transcript variant 1	Telomeres	Elderly	NM_173156
SNCA	Homo sapiens synuclein, alpha (non A4 component of amyloid precursor) (SNCA), transcript variant NACP140	Disease	Elderly	NM_000345
SPATA1 SP2	Homo sapiens spermatogenesis associated 1	Hormone	Juvenile	NM_022354

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
SPINK5L3	Homo sapiens serine PI Kazal type 5-like 3	Affymetrix	Juvenile	XM_376433 Replaced by NM_001040129
SPINKa	Homo sapiens cDNA FLJ30191 fis, clone BRACE2001313	Affymetrix	Juvenile	AK054753
SPINKb	Homo sapiens cDNA FLJ30191 fis, clone BRACE2001313	Affymetrix	Juvenile	AK054753
SPP1	Homo sapiens secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1), transcript variant 1	Bone	Elderly	NM_001040058
SPTRX-1	Homo sapiens thioredoxin domain containing 2 (spermatozoa) (TXNDC2)	Hormone	Juvenile	NM_032243
SPTRX-2	Homo sapiens thioredoxin domain containing 3 (spermatozoa) (TXNDC3)	Hormone	Juvenile	NM_016616
SRC	Homo sapiens v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian), transcript variant 1	Oncogene	Elderly	NM_005417
SRPX	Homo sapiens sushi-repeat-containing protein, X-linked	Affymetrix	Juvenile	NM_006307
SST	Homo sapiens somatostatin	Hormone	Juvenile	NM_001048
STAF42	wi67g12.x1 NCI_CGAP_Kid12 Homo sapiens cDNA clone IMAGE:2398438 3-	Affymetrix	Newborn	AI760812
STK16	Homo sapiens serine/threonine kinase 16, transcript variant 1	Affymetrix	Elderly	NM_003691
TBC1	Homo sapiens TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1, mRNA (cDNA clone IMAGE:5211948), with apparent retained intron	Affymetrix	Juvenile	BC028196

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
TEKT2	Homo sapiens tektin 2 (testicular)	Hormone	Juvenile	NM_014466
TEP1	Homo sapiens telomerase-associated protein 1	Telomeres	Elderly	NM_007110
TERF2	Homo sapiens telomeric repeat binding factor 2	Telomeres	Elderly	NM_005652
TERT	Homo sapiens telomerase reverse transcriptase, transcript variant 1	Telomeres	Elderly	NM_198253
TFAP2BL1	Human DNA sequence from clone RP3-336H9 on chromosome 6p12.1-21.1, complete sequence	Affymetrix	Juvenile	AL031224
TINF2	Homo sapiens TERF1 (TRF1)-interacting nuclear factor 2	Telomeres	Elderly	NM_012461
TNFAIP3	Homo sapiens tumor necrosis factor, alpha-induced protein 3	Apoptosis	Elderly	NM_006290
TNFRSF11A	Homo sapiens tumor necrosis factor receptor superfamily, member 11a, NFKB activator	Bone	Elderly	NM_003839
TNFSF10	Homo sapiens tumor necrosis factor (ligand) superfamily, member 10	Apoptosis	Elderly	NM_003810
TNIP1	Homo sapiens TNFAIP3 interacting protein 1	Apoptosis	Elderly	NM_006058
TNKS1BP1	Homo sapiens tankyrase 1 binding protein 1, 182kDa	Telomeres	Elderly	NM_033396
TP53	Homo sapiens tumor protein p53 (Li-Fraumeni syndrome)	Tumor Suppressor Genes	Elderly	NM_000546
TP53BP1	Homo sapiens tumor protein p53 binding protein, 1	Tumor Suppressor Genes	Elderly	NM_005657

Candidate Gene	Gene Description	Category	Target Age Group	NCBI Accession ID Number
TP53BP2	Homo sapiens tumor protein p53 binding protein, 2, transcript variant 2	Tumor Suppressor Genes	Elderly	NM_005426
TP53I3	Homo sapiens tumor protein p53 inducible protein 3, transcript variant 1	Tumor Suppressor Genes	Elderly	NM_004881
TP73	Homo sapiens tumor protein p73	Disease	Elderly	NM_005427
TPST1	Homo sapiens tyrosylprotein sulfotransferase 1	Affymetrix	Elderly	NM_003596
TRPC1	Homo sapiens transient receptor potential cation channel, subfamily C, member 1	Affymetrix	Juvenile	NM_003304
TSLL2	Homo sapiens cell adhesion molecule 4 (CADM4)	Affymetrix	Juvenile	NM_145296
TUFT1	Homo sapiens tuftelin 1	Affymetrix	Juvenile	NM_020127
UNQ501	Homo sapiens MBC3205	Affymetrix	Elderly	NM_198536
VSIG2	Homo sapiens V-set and immunoglobulin domain containing 2	Immunology & Interferons	Elderly	NM_014312
WHSC1L1	ny99e02.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:1286426 3-	Affymetrix	Newborn	AA741074
WRN	Homo sapiens Werner syndrome	DNA Damage & Growth Arrest	Elderly	NM_000553
XTP3TPA	Homo sapiens XTP3-transactivated protein A	Affymetrix	Elderly	NM_024096

APPENDIX E: CANDIDATE GENE PRIMER SEQUENCES FOR RT-PCR

Table 26: Candidate Gene Primer Sequences for RT-PCR.

NEWBORN CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
AFP	tcctcagcttgctgtctcag	gctgccatttttctggtgat	NM_001134
APOE (1)	ggtcgcttttgggattacct	tccagttccgatttgtaggc	M12529
APOE (2)	aggaagatgaaggttctgtg	ctcagttcttgggtgacttg	NM_000041
ATF7IP2	ccagtaaatgacctgcgaca	aaggcaaggaaagcagaaca	AV7169647
AUF1 hnRNPD (p37)	F1 aacgaggaggatgaaggga	R1 ccataaccactctgctggtca	NM_001003810
CHR1orf28	gcctgaatcttgattcccatt	gggatggtctagtgcaaagg	AA447464
COL1A2	tggagtccgaggacctaatg	gcaagaccagcatgaccttt	NM_000089
DUSP6	ctgtcccagtttttccctga	tcacagtgactgagcggcta	BC005047
FACL6	tgtaggcctagccccatgta	tgtgcttcatacatttgcacag	AV727634
FKLF	tgcagccacacctgaactac	tgtgtcggatcacgctagtc	AF272830
FLJ20344a	agtggcagcaactggactct	tcaacttgccagacttctgc	NM_017776
FLJ20344b	ctcccaaagtgctgggatta	tgcaaattgccaacatcact	NM_017776
FLJ21901	gacccgctagttgaagcact	acgttggcctcagaagaatc	NM_024622
FLJ30658	cctgggaaatgccaaaaata	attttgaagccaggtgatgc	AW770868
FLJ35954	tcaatgcaatagcaacttcctc	tccaattgtcccagtttgaa	R66534
HBE1	aacatggacaacctcaagcc	cacctgcaaactggaagagaa	NM_005330
HBG1	acttccttgggagatgccac	aaagcctatccttgaaagctctga	NM_000559
HBG1n1	gaaagctctgaatcatccaggtg	gggcaaggtgaatgtggaag	N/A
HBG1n2	agtgagctcagtggcatctc	gggcaaggtgaatgtggaag	N/A
HBG2	acttccttgggagatgccat	gcctatccttgaaagctctgc	NM_000184
HBG2n2	ctggaggacagggcaaagg	gggcaaggtgaatgtggaag	N/A
HBG2n3	ggcagtgagctcagtgcagttc	cagctttggcaacctgtcct	N/A
HBZ	catgtctctgaccaagactgaga	ggatacgaccgataggaacttgt	NM_005332
ITSN2	gggagtgctagcaagtctgg	atgactggcaggaaaccatc	NM_006277
KIAA0276	gactttgcacgggaaaagg	actggaaaaaggggccag	D87466
KIAA1265	cagggtggacatgatcacag	ggcttgagttgaagccagtc	AB033091
KITLG	gctttgcttttggagcctta	tgtggtctgtcactccagaca	NM_000899
LOC151194	tacctggagatgggagctgt	tgctacttttcgatccgtga	NM_145280
LZTFL1	gggctagtgtggccttcag	tgcttggcatagttggtttt	NM_020347
MIF	ttcatcgtaaacaccaacg	ttgctgtaggagcggttc	L19686
PITPNC1	cttcagcagtggcagtggta	tgttgggaaattttcagatgc	AI676095
PPAT	cagaggcaataccatctcacc	cccttcttgtacagatgaaacca	U00238
RaI	cgtggtggtttaaacactgg	gcagcctgttgatcttttgg	AW003297
RaIGPS2	catgcacttatggcagtggt	agggaatgcaaggtgtcatc	NM_018037
STAF42	cccaaaaggtttatttgtca	tgcttggtaattctccagtt	AI760812
WHSC1L1	caacagaaacgcttttataagataca	gtgattttgccagctggttc	AA741074

JUVENILE CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
ACTA2	gtggttctggtttgcctgat	ctggccctgtaacaccagat	AK093340
ADAM12	gtgcttttggctaccacaca	agttcttccccaccgagttt	AU145357
ADAM12a	gtgcttttggctaccacaca	aattataacagtgaacaagattggtgtt	AU145357
Art3a	tgcagccattatgagtgtgc	tatttgggtgggttcaagga	U47054
Art3b	actttgggggcaaaagaag	acttcagccttcacctggaa	U47054
ASL	aacatgggacaggctctcag	tagtcccacacgcagatcac	NM_000048
BCKDHA	cttgagtgccccatcatctt	cctcctttgtggcgttgtat	NM_000709
c5229134	acgtcactgtccaactcgtg	ctgtgacctggaggttggtt	BC037976
c5286506	ctcacagacacacccagaaac	aaaattccaggtgccaacag	BC043160
CABP7	ggctgctctacgacaccttc	ccaggtggcgtctacttcat	NM_182527
CABYR	gagctgttctcaaaaccaac	tgtcctcgtctgtgtctgta	NM_012189
CD28	gaaacacctttgtccaagtc	ggggagtcatgttcatgtag	NM_006139
CD200	ggggactgtgaccgacttta	tcaggtcctttggagaatgg	NM_001004196
CFIX	atgcattctgtggaggctct	cagttccagaagggcaatgt	NM_000133
CGI-96	agagagccaccctgtgaaga	ctggtcatatgcctccatga	AL157851
COL6A1a	ctggccctatcggacctaa	aagccctcggtgccattt	M20776
COL6A1b	gatgggagaaaggggagaag	gggtgcaatgtcgttgttatc	M20776
CXorf22	tgttttcgggggacagttag	tagcttcaacgcgtttcctt	NM_152632
CYP17A1	tgatggacgcctttatcctc	cataaaggaaggccaggaca	NM_000104
CYP1B1	gtggagaccaccacctctgt	gctgaaacccacattctggt	NM_000102
CYP7B1	aggcaagatgtcctggagaa	gggtgccgcagaagataata	NM_004820
DHEA	ggtttgaccacattcatgg	gggccactgtgaagtgattt	NM_003167
DNCL2A-1	atcggtcggaaatggca	cgaattcgaaggaaggtgag	NM_014183
DNCL2A-2	ccaggaggtcaaggctacag	gatgggaatgccttctgtgt	NM_177953
DNCL2A-3	agggaatcatcgtcgtgaac	agagcaagaagtgcccaaaa	NM_177954
DNPEP	gtcggtgtggagacctatgg	tatttcgctgcagatggatg	NM_012100
E2IG2	tgtacagaggatccccaacc	ggtcctcctcctcatcgtct	NM_016565
ERBP	ggttgccattgaggaagaaa	tgctgctgcttgtcaacttc	NM_014597
ESR1	gtgcctggctagagatcctg	agagacttcagggtgctgga	NM_000125
ESR2	tggagtctggtcgtgtgaag	gtcggcacttctctgtctcc	NM_001437
FKBP11	cctatggaaaacggggattt	catccctaccagaggcaaaa	NM_016594
FLJ11078	tcctcgatgttgtgctgact	agtccaggtccagtgtcacc	NM_018316
FLJ22175	acatctgcagtgtcgtctcg	aggcttgtcagtgccttgtt	NM_025161
FLJ22672 PAQR6	cacctgcaccagttctttgt	aagaggaagccagtgagcag	NM_024897
FLJ35119	aaacagcgctgctatttgct	ttgagggtgggtactggaag	NM_175871

JUVENILE CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
FLJ35982	gccctgaatctcaggcact	ggacagggaaggggatttta	AK093301
FLJ35982a	tcaggcactggaaggttacc	ggacagggaaggggatttta	AK093301
FLJ37440	agccttgtcaaaatggtggt	ctgcccgtagagctcacact	NM_153214
FLJ38745	ttgtgtggatgacgtcctgt	cagcctccatgaggttgatt	AK096064
FLJ43159	gcctgcattgccttatgaat	cagctgctttacccaggaac	AI972146
GAL	tcattcagcgacaagaatgg	tgcataaattggccgaagat	NM_015973
GFPT2	cagggatgacgtttgctttt	gatctcaagccacggatgat	NM_005110
GGT1	gtgttctgccgggatagaaa	caggtcctcagctgtcacaa	NM_005265
GHRH	aattggagagcatcctggtg	ccagttgcattttggctaca	NM_021081
GLO1	atgcgacccagagttaccac	ttcaatccagtagccatcagg	NM_006708
GNRH1	ctactgacttcgtgcgtgga	cttctggcccaatggattta	NM_000825
GNRH2	gatccccagaatgcccttag	cttcctgtgaagggaccact	NM_001501
GNRHR	ctggcctggatcctcagtag	ggcagctgaaggtgaaaaag	NM_000406
GPR54	ctcgctggtcatctacgtca	actcatggcggtcagagtg	NM_032551
GRIN1 NR1-1	cgggatcttcctgattttca	ggatggtactgctgcaggtt	NM_000832
GRIN1 NR1-2	cgggatcttcctgattttca	caccccggtgctctg	NM_021569
GRIN1 NR1-3	cgggatcttcctgattttca	tgtctttggaggacctacgc	NM_007327
GRIN2A	caagtgggagaaccatacgc	cattcatcccctcattggtt	NM_000833
GRIN2B	gcatgcctacatgggaaagt	tctccaaagagctgcaggat	NM_000834
H17	aaggtccagtccttgggagt	ctcggctccacaggtagctt	NM_017547
HOMER3	ccaggaagtgaaggaagcag	gtcctgcagtgcgaaaaact	NM_004838
HRG	gcccgaaaaaccttgtcata	ctagatccatggggcttgaa	NM_000412
HTR1E	cctcccaaagtgctggaat	tgtagcctcgaaggtttctca	NM_000865
HTR7	ccctccaactacctgatcgt	aagccagacggagagaatca	NM_000872
INHA	ctctgagcccgaggaagag	gagctattggaggctgctgt	NM_002191
ITIH4	ggacctcctgatgttcctga	agggtctgagagcaggttca	NM_002218
KIAA0894	ggtgaactcttttcgcaagc	agagcacacacagtccaacg	NM_014896
KIAA2022	cagccaacggagaaaacact	gctctgcatacagggcttct	AB095942
KISS-1	tggcagctactgcttttcct	cagtagcagctggcttcctc	NM_002256
LATS1	gctgtcgatgtggagacaga	ggttgtcccaccaacatttc	NM_004690
LEP	ggctttggccctatcttttc	accggtgactttctgtttgg	NM_000230
LHB	gtcaacaccaccatctgtgc	ggaagaggaggcctgagagt	NM_000894
LHCGR	aggctaattgccacgtcatc	gggtgtcttgggtaagcaga	NM_000233
LOC152274	aggaggagagaagggagcag	tcaactcctcgggaatgaac	AK056398
LOC284242	gctgaggagagggaagtgaa	gtggctctcagctctgctct	BC035844

JUVENILE CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
MGC20460	cagcagcccaagaacataca	actgttgggaacaggtccag	NM_053043
MGC39650	catctacccttttcgcctctg	agatcatctgccccacactc	AL137531
NALP14	gtcttgggtgatggtggagt	agatgcgtcaggctcttgtt	NM_176822
NDE1	gagtccaaactcgcttcctg	ccagcagtacgaggagaagg	NM_017668
NTS	gcatgctactcctggctttc	ccaagagggaacatgtgctt	NM_006183
OSGEP	attggtgggtgtgaaccact	cctggacttgggtcgttaga	NM_017807
OXTR	ttcttcgtgcagatgtggag	ggacgagttgctctttttgc	NM_000916
PAQR6	ggggctcttctgggaaaata	agagcctcccctcaccag	NM_024897
PDE6D	gacctgtctgtccctggtgt	ggtgctgcctctatcaagga	NM_002601
PGR	gtcagtgggcagatgctgta	tgtgagctcgacacaactcc	NM_000926
PLEKHA8	ggcatcatgttatgctgtgg	gcttcagtcgctgagttcct	NM_032639
POMC	aggacctcaccacggaaag	gaagtggcccatgacgtact	NM_000939
PPOX	tctagccatggacagtctct	ctctagacctccacgaagtg	NM_000309
PRL	tccataacctctcctcagaaa	ataccacgtacttccgtgac	NM_000948
PTH	gggtctgcagtccaattcat	gcttcttacgcagccattct	NM_000315
PTMS	ctgaagagagctgccgaaga	aggctggggagaaagaagag	NM_002824
RAPA-2 TRERF1	ggctcttcagcaatgtcctc	cagggcttccataccagtgt	AJ277276
RARA	gggagctcattgagaaggtg	gtccgagaaggtcatggtgt	NM_000964
REA	gagctgagctttagccgaga	gggttcttgctcagtgcttc	NM_007273
SH3GL1	ctggcagaggtgaaggactc	gactcacctgctcgatgtca	NM_003025
SHBG	tcttggctcagtctccacct	ctcaagaccaccctggacat	NM_001040
SLC39A4	ttcgtggactttgtgttcca	acacactggagctgttgctg	NM_017767
SPATA1 SP2	caacctgttctttcttcagg	ttttgttaaaacctcctcca	NM_022354
SPINK5L3	tttggcacacacacacacac	agccttgagaagagctgctg	XM_376433
SPINKa	cagaagcagaagcccctatg	gccttcctctctgtcagtgg	AK054753
SPINKb	tgtgcttgcttccttgtcac	atctttgaggtcgtccatgc	AK054753
SPTRX-1	acagagagggaaaaccaact	tggtttcttctgaggacttg	NM_032243
SPTRX-2	gagcaatgcaacctttattc	tgcaatttttctctcctcat	NM_016616
SRPX	tcaagtgcccaagtgtgaag	ttctctggggcattgagttt	NM_006307
SST	cccagactccgtcagtttct	ccatagccgggtttgagtta	NM_001048
TBC1	tcacaacagtcatgacccaag	ggccactgggatgaactaga	BC028196
TEKT2	tgacacagatgaaggagtca	acagcctctgtcgatctcta	NM_014466
TFAP2BL1	ctagagaccaggctgccatc	gcagtgggttcagggagtag	AL031224
TRPC1	tgcttaccaaactgctggtg	tggtgagggaatgatgttga	NM_003304
TSLL2	cggataacggcacctacact	aaccgacgtctgagcctcta	NM_145296
TUFT1	agaggaacttcggagcaaca	gctcttgagcatgtcatcca	NM_020127

ADULT CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
AUF1 hnRNPD (p45)	F2 cccacgacactctgaagcag	R2 tccctggttccagttttgac	NM_031370
HBA1	gttaagggccacggcaag	ccaaggggcaagaagcat	NM_000558
HBA2	gttaagggccacggcaag	cagcgggcaggaggaac	NM_000517
HBB	tcctttggggatctgtcca	aaggaacctttaatagaaattggacag	NM_000518
HBD	ctgaggagaagactgctgtcaa	gaattccttgccaaagttgc	NM_000519
HBQ	cggctcctcacaagtcaga	agttcagcggtactcggaaac	NM_005331
ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
ABL1	gagggcgtgtggaagaaata	agtccaggaggttcccgtag	NM_007313
ACD	cagctcaatgctgtgcatct	ggtaccactttcctcggatg	NM_022914
ACTN3	gattcggctttgctacagga	agctggtcaatggtctccag	NM_001104
AGGF1	cacagaacggctgtaccaga	agattgaccaaggagcatgg	NM_018046
AIF1	ttggagtccccaagactcac	ccttcaaatcagggcaactc	NM_001623
AKT1	atggcaccttcattggctac	aaggtgcgttcgatgacagt	NM_005163
AMID	ggggatagacctgaagaacca	aatctctgctgccatctcca	NM_032797
ANKH	ctgtgcctgggctactacaa	ggccgactgattctctgtgt	NM_054027
APEX1	caaacctgccacactcaaga	gctgttaccagcacaaacga	NM_001641
ARMC7	gagaatgagaccctggtgga	agacagcaccgtctcctcat	NM_024585
ATPAF2	gagatcagctcctccaccag	actcaatgttgccccacttc	NM_145691
BAX-(all)	tgatggacgggtccgg	cccctgtcttcatgatctgc	NM_138764
BAX-a/d	aactggtgctcaaggccc	ggcgtcccaaagtaggaga	NM_138761
BAX-all(e)	tctgacggcaacttcaactg	ggaggaagtccaatgtccag	NM_138764
BAX-b	tctgacggcaacttcaactg	cactgtgacctgctccagaa	NM_004324
BAX-d	ccctttttgcttcagggga	ggaggaagtccaatgtccag	NM_138763
BAX-e	tctgacggcaacttcaactg	aatcgcttgaacccaggag	NM_138764
BAX-s	tctgacggcaacttcaactg	aaagatggtcacggtccaa	NM_138765
BCL2A1	ggcatcattaactggggaag	tccagccagatttaggttcaa	NM_004049
BGLAP	ggcagcgaggtagtgaagag	agcagagcgacaccctagac	NM_199173
BIRC5	ggaccaccgcatctctacat	gtctggctcgttctcagtgg	NM_001012271
CAMK2D	actatcaaccctgccaaacg	ccccattgttgatagcttcg	NM_172127
CASP2	agactgatcgtgggggttgac	caggaacctcgtttggtgtt	NM_032982
CBL	tctaatgccagctcctcctt	ggccatctcgatgttgttct	NM_005188
CCL5	tacaccagtggcaagtgctc	tgtactcccgaacccatttc	NM_002985
CCM2	tgtttacacggagtccacca	accacccacatccacagat	NM_001029835
CCND1	tcctctccaaaatgccagag	tgaggcggtagtaggacagg	NM_053056

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
CD28	cggaccttctaagccctttt	atagggctggtaatgcttgc	NM_006139
CD86	agacgcggcttttatcttca	ttaaaaacacgctgggcttc	NM_175862
CDC2	ccatggggattcagaaattg	ccattttgccagaaattcgt	NM_001786
CDC25C	ggcacctgattggtgatttt	ctggaacttccccgacagta	NM_022809
CDKN1A	ggaagaccatgtggacctgt	ggattagggcttcctcttgg	NM_078467
CDKN1B	ccggctaactctgaggacac	cgagctgtttacgtttgacg	NM_004064
CDKN2C	acgtcaatgcacaaaatgga	cgaaaccagttcggtctttc	NM_001262
CIITA	gatgtggaagacctgggaaa	cacccaggtcagtgatgttg	NM_000246
CLEC2	tgatggctttgattctgctg	acaggggctgcatttatgac	NM_016509
CLEC2a	agctctcgtctccgttgg	cgctttgctaattgttgcag	NM_016509
CLEC2b	gcacaggaactctgcaacaa	gcctgaagaacccatagcag	NM_016509
COL1A1 CTx	atggctctcctggcaaagat	atcaccaggttcgcctttag	NM_000088
CTBP1	ccttcctggtgaacacagc	ggctgtcagatggtccttgt	NM_001012614
CTSB	ggccgagatctacaaaaacg	gccaccacttctgattcgat	NM_147780
CTSK	ccttgaggcttctcttggtg	tccacagccatcattctcag	NM_000396
CTSL	acagtggaccaagtggaagg	tgggcttacggttttgaaag	NM_001912
CYTBC2	gatggagcggttctggaata	ccagacacagggacttcaca	D49737
DDB2	cgatggaaactcagggaaga	aaatcaccacctctgcttgc	NM_000107
DYRK2	gccatgttaaccaggaaacc	cgacatgcaggtgatcattc	NM_006482
E2F1	agctggaccacctgatgaat	ctcagggcacaggaaaacat	NM_005225
ECGF1	acaaggtcagcctggtcctc	ctctgacccacgatacagca	NM_001953
ELAVL1 HuR	acaaaaacgtggcactcctc	gccccaggttgtagatgaaa	NM_001419
EMD	gccgcctcctcttatagctt	tgatgctctggtaggcactg	NM_000117
EREG	cgtgtggctcaagtgtcaat	agtgttcacatcggacacca	NM_001432
ERF	gggaaacggttcacctacaa	agatgaagagcaggctggtg	NM_006494
FLJ20245	gctgctcctggagtcttgg	gctcctgggacagatactcg	NM_017723
FLJ20421	gcatttaaagccatggagga	ctgaaaccatggggagagaa	BF674724
FLJ35984	accaggggtccatcctctac	ggaggtgctgggtttcataa	AK093303
FLJ38628	ctcaaggacagaggccagag	cagggccacaaataggaaga	NM_152267
GADD45A	ggaggaagtgctcagcaaag	atctctgtcgtcgtcctcgt	NM_001924
GADD45B	tgctgtgacaacgacatcaa	tttgtttgtggcagcaactc	NM_015675
GSTP1	gacctccgctgcaaatacat	ggctaggacctcatggatca	NM_000852
HIC2	ctgctgctcacatggtgtct	gatgacgtcacacaggaagc	NM_015094
HIF1A	tccatgtgaccatgaggaaa	ccaagcaggtcataggtggt	NM_001530
HPCAL4	caactgggcctttgagatgt	tggtcgtccttatcctggtc	NM_016257
HRAS	gagggcttcctgtgtgtgtt	agccaggtcacacttgttcc	NM_176795

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
HTATIP	catcctccaggcaatgagat	agtagcccacgatgtggaag	NM_182710
IFNG	agatgaccagagcatccaaaa	cagttcagccatcacttgga	NM_000619
IGF1	tggatgctcttcagttcgtg	cctgcactccctctacttgc	NM_000618
IGF2	acaccctccagttcgtctgt	cggaaacagcactcctcaac	NM_000612
IGFBP3	acagccagcgctacaaagtt	ggctgcccatacttatccac	NM_001013398
IGFBP5	tgcacctgagatgagacagg	gaatcctttgcggtcacaat	NM_000599
IL1A	aatgacgccctcaatcaaag	ccgtgagtttcccagaagaa	NM_000575
IRF1	ccaggctacatgcaggactt	gtaggtacccctttcccatcc	NM_002198
KL	aatggctggtttgtctcagg	tgtaacctctgtgccactcg	NM_153683
KLF13	gatcctagcggacctcaacc	attcccgggtggaagttg	NM_015995
LASS5	aaaatccaatgctggtttcg	ccaatagaaggccaattcca	NM_147190
LMNA (norm)	ggtggtgacgatctgggct	ccagtggagttgatgagagc	NM_170707
LMNA (RT)	gtggaaggcacagaacacct	gtgaggaggacgcaggaa	NM_170707
LMNA (spec)	gcgtcaggagccctgagc	gacgcaggaagcctccac	NM_170707
LOH11CR2A	ggcaccactccagaacattt	tcacccggaaatcacatttt	BC001234
MAD1L1	gagcagatccgttcgaagtc	gcatccaagttctgctgaca	NM_003550
MCPH1	agcccagagtgaacatgagc	aggtccttaaagccgtcaca	NM_024596
MDM2	ggtgctgtaaccacctcaca	tttttgtgcaccaacagacttt	NM_002392
MEPE	aactaagcaaagctgtgtgg	attctcactggcttcagaaa	NM_020203
MET	agcctgattgtgcatttcaa	gatgattccctcggtcagaa	NM_000245
MGC14288	gggaagttgcgtagacagtg	agctagctgcttgccagttg	NM_032901
MLL	taagcccaagtttggtggtc	cttctgcaggtaggctttgg	NM_005933
MMP-13	aacatccaaaaacgccagac	atgcagcatcaatacggttg	NM_002427
MMP-14	cactgcctacgagaggaagg	tcccttcccagactttgatg	NM_004995
MMP-9	gacaagctcttcggcttctg	gccattcacgtcgtccttat	NM_004994
MS4A4A	ggaatgaaattacgtctttggaa	cctgatgcagccagtacaga	NM_024021
MS4A4Aa	tctgtactggctgcatcagg	gccatgtgagaatgtgatgg	NM_024021
MS4A4Ab	aggagagagattcgagcacct	ggcagtcagaatctgcacaa	NM_024021
MT1X	tcctgcaaatgcaaagagtg	acagctgtcctggcatcag	NM_005952
MYC	cctaccctctcaacgacagc	ctctgacctttgccagga	NM_002467
NBN	gaaaaaggccaaggatggat	gccagatggatttctggaag	NM_001024688
NMI	cgcgtggactatgacagaca	gcccgttgaaagtgaatgtt	NM_004688
NPPB	accgcaaaatggtcctctac	gttgaggaaaaagccccttg	NM_002521
NRAS	gcgaaggcttcctctgtgta	agttcgtgggcttgttttgt	NM_002524
OGG1	atggggcatcgtactctagc	cgatgttgttgttggaggaa	NM_016819
OPG TNFRSF11B	ggcaacacagctcacaagaa	gtgtcttggtcgccatttttt	NM_002546

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
OPGL RANKL TNFSF11	gcttgaagctcagccttttg	cgaaagcaaatgttggcata	NM_003701
OSM	agctgctcgaaagagtaccg	ctgctctaagtcggccagtc	NM_020530
PDCD1	aaggcgcagatcaaagagag	aatccagctccccatagtcc	NM_005018
PDCD10 CCM3	tgaagctgagaccacatcca	tgccatacgaagaagggact	NM_007217
PDCD11	gggcaagaagagtgtcaagc	gtggcagaaaagctctggtc	NM_014976
PDCD1LG2	atccaacttggctgcttcac	aagtgcaaatggcaagctct	NM_025239
PDCD2L	ctggtcgtgcaggtgtattg	gaaggcccctcctcagtatc	NM_032346
PDCD4	tggattaactgtgccaacca	tctcaaatgccctttcatcc	NM_145341
PDCD5	cttgaggcgctgaggagac	ccgactgatccagaacttgg	NM_004708
PDCD6	ctccgggatgatcgataaga	tccatgttgtgctgctcttc	NM_013232
PDCD6IP	ctgttgggaccctcagtctt	ttctgctgttttgccaggat	NM_013374
PDCD7	tgaagtgtgtgcaggaggtg	gtcgctgaagatgatgcgta	NM_005707
PIK3CA	cagacgcatttccacagcta	gcaaatggaaaggcaaagtc	NM_006218
POLA1	ccatcacagttttgcattgg	cagtgaggagctttgcacac	NM_016937
POLA2	cgaaagccaggcatagtacc	ggggctacgagttgacacac	NM_002689
POLB	attcggcaggatgatacgag	ccaattcgctgatgatggtt	NM_002690
POLD1	ggtgcagagctacgagaagg	atgaagagtcccggatgttg	NM_002691
POLE1	tggcatttgacattgagacg	gtttggtctcctggacgtgt	NM_006231
POLE2	ttgaacgatctgtggtggaa	aaatttggtgcagggtggt	NM_002692
POLE3	agaggcccgaggacctaaa	agcacatcactggcattcag	NM_017443
POLG	tgaggccaagatggagaact	tacgtttatgggcgttcctc	NM_002693
POLH	tggactaaacaagcccaacc	gttgcctgggtttaactgga	NM_006502
POLI	cagttgctcagcgtatccaa	aaggaatagggcactgacga	NM_007195
POLK	ccatgccaggatttattgct	ggatcgttcatgctcactca	NM_016218
POLM	ttccccactttggagaacac	gtaccaccggtcagcagtct	NM_013284
POLN	ccaagcacccaattcagatt	acaccaccttcttggtttgc	NM_181808
POLQ	gccttcaggactggactctg	agtagaagttgccgccaaga	NM_199420
POLR3F	tgcaaaagaaggcacagttg	aaaattcgagccactctgtc	NM_006466
POLR3K	atcgtggaggagggacaac	caccaagcacatcatccact	NM_016310
POLS	cccaccacttccagaacact	gctttcaaagacgcagttcc	NM_006999
POT1	tgggtattgtacccctccaa	ttgatgaagcattccaacca	NR_003102
PPARD	aagtggcagaggcagaag	ctgcgctcacacttctcgta	NM_006238
PRDX5	cgctcagcgggctatatact	aaagatggacaccagcgaat	NM_012094
PRKCA	caggatgatgacgtggagtg	gttccttgcacatcccaaag	NM_002737
PTGER4	ctggtggtgctcatctgct	tcacagaagcaattcggatg	NM_000958

ELDERLY CANDIDATES			
Candidate Gene	Forward Primer Sequence 5' → 3'	Reverse Primer Sequence 5' → 3'	NCBI Accession Identification Number
PTPN18	ccagatgatcccacctgact	gaagagggcatcgtcgtaga	NM_014369
RAD50	cttggatatgcgaggacgat	ccagaagctggaagttacgc	NM_005732
RAF1	ggctggtagctgactgtgtg	ccggttgatcttcggtagag	NM_002880
RANK	ctctgatgccttttcctcca	agctggcagagaagaactgc	AF018253
RB1	aggaccgagaaggaccaact	cagacagaaggcgttcacaa	NM_000321
RBL1	ttgatggcttgttgtttgga	tgtttcaccatgtcccttga	NM_002895
RBL2	agaacctggaaagggcagat	tggggagctgtacctatcgt	NM_005611
RELA	ccacgagcttgtaggaaagg	ctgatagcctgctccaggtc	NM_021975
RUNX2	cggaatgcctctgctgttat	atgcgccctaaatcactgag	NM_004348
SEMA4A	tctgctcctgagtggtgatg	aaaccaggacacggatgaag	NM_022367
SLC20A1	ctatgcctgcacagttggaa	accagacgataagggcacag	NM_005415
SMG5	ctgcagttcaacccagaggt	aggtagggagacatggctga	NM_015327
SMG6	tgcctccactactgcaaaga	ggcatcttccgtgctacact	NM_017575
SMG7	caggagtcttccgtccagag	tgagagagaatccggtgagg	NM_173156
SNCA	aaaaccaaggagggagtggt	cccaactggtccttttgac	NM_000345
SPP1	gccgaggtgatagtgtggtt	attcaactcctcgctttcca	NM_001040058
SRC	ggctacatccccagcaacta	tgcggatcttgtagtgcttc	NM_005417
STK16	gggttccatgaatcaagcat	ccctttggaacaccatgtc	NM_003691
TEP1	gccgcactgtcttggtctat	ggagcttgatggcagtcttc	NM_007110
TERF2	gaccttccagcagaagatgc	cctgtgcaccagacagagtc	NM_005652
TERT	gcaaactctttggggtcttg	gggttcttccaaacttgctg	NM_198253
TINF2	tcctgaaagccctgaatcac	ctgcatccaactcagcacat	NM_012461
TNFAIP3	atagaaatccccgtccaagg	tgggcgtttcacattttaca	NM_006290
TNFRSF11A	catgtttacttgcccggttt	cctgacagacaccaccttga	NM_003839
TNFSF10	gagtatgaacagcccctgct	tccttgatgattcccaggag	NM_003810
TNIP1	tgagcaatggcaacaaagag	gctccagcatcttcaccttc	NM_006058
TNKS1BP1	ggaggggccagtaaagtctc	ctcttatcaggcgggtgaag	NM_033396
TP53	gcgcacagaggaagagaatc	cctcattcagctctcggaa	NM_000546
TP53BP1	cccatacttgggagtggaaa	cctcacttcgagcctcattc	NM_005657
TP53BP2	tccttggtcattcaggcttc	cggacgcactttcttctctt	NM_005426
TP53I3	gcttcaaatggcagaaaagc	aacccatcgaccatcaagag	NM_004881
TP73	gaccgaaaagctgatgagga	tcagctccaggctctctttc	NM_005427
TPST1	cccacctaactacggaaaacc	aagaggctcctggttctgct	NM_003596
UNQ501	atgcaaatgtgggtgacctt	aggctcaggaacagcaggta	NM_198536
VSIG2	tgcgtcttggaacttttcct	cccctctctttctggaacct	NM_014312
WRN	ggactttggtccacaagcat	tctttggtgcccgaagatac	NM_000553
XTP3TPA	cctccatgctgagtttgctg	atgccaccaggtagatgagg	NM_024096

APPENDIX F: CANDIDATE GENE RT-PCR RESULTS

Table 27: Candidate Gene RT-PCR Results.

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
ABL1	Elderly	Literature		Expressed in All Ages
ACD	Elderly	Literature		No mRNA Detected
ACTA2	Juvenile	Affymetrix		No mRNA Detected
ACTN3	Elderly	Literature		No mRNA Detected
ADAM12	Juvenile	Affymetrix		Same Size mRNA/DNA
ADAM12a	Juvenile	Affymetrix		Same Size mRNA/DNA
AFP	Newborn	Literature	Fetal Liver	
AGGF1	Elderly	Affymetrix	Elderly	
AIF1	Elderly	Literature		Expressed in All Ages
AKT1	Elderly	Literature		Expressed in All Ages
AMID	Elderly	Literature		Expressed Sporadically
ANKH	Elderly	Literature		No mRNA Detected
APEX1	Elderly	Literature		Expressed in All Ages
APOE (1)	Newborn	Literature		No mRNA Detected
APOE (2)	Newborn	Literature		Expressed Sporadically
ARMC7	Elderly	Affymetrix		Expressed Sporadically
Art3a	Juvenile	Affymetrix		No mRNA Detected
Art3b	Juvenile	Affymetrix		No mRNA Detected
ASL	Juvenile	Affymetrix	Juvenile	
ATF7IP2	Newborn	Affymetrix		Expressed in All Ages
ATPAF2	Elderly	Affymetrix		Expressed Sporadically
AUF1 hnRNPD p37	Newborn	Literature		Expressed Sporadically
AUF1 hnRNPD p45	Adult	Literature		Expressed Sporadically
BAX-(all)	Elderly	Literature		Expressed in All Ages
BAX-a/d	Elderly	Literature		Expressed in All Ages
BAX-all(e)	Elderly	Literature		Expressed in All Ages
BAX-b	Elderly	Literature		Expressed Sporadically
BAX-d	Elderly	Literature		Expressed Sporadically
BAX-e	Elderly	Literature		Expressed in All Ages
BAX-s	Elderly	Literature		No mRNA Detected
BCKDHA	Juvenile	Affymetrix		Expressed in All Ages
BCL2A1	Elderly	Literature		Expressed in All Ages
BGLAP	Elderly	Literature		Expressed Sporadically
BIRC5	Elderly	Literature		Expressed in All Ages
c5229134	Juvenile	Affymetrix		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
c5286506	Juvenile	Affymetrix		Same Size mRNA/DNA
CABP7	Juvenile	Affymetrix		No mRNA Detected
CABYR	Juvenile	Literature		No mRNA Detected
CAMK2D	Elderly	Literature		Expressed in All Ages
CASP2	Elderly	Literature		Expressed in All Ages
CBL	Elderly	Literature		Expressed in All Ages
CCL5	Elderly	Affymetrix		Expressed in All Ages
CCM2	Elderly	Literature		Expressed in All Ages
CCND1	Elderly	Literature		Expressed Sporadically
CD200	Juvenile	Literature		Expressed Sporadically
CD28	Juvenile	Affymetrix		Expressed in All Ages
CD28	Elderly	Literature		Expressed in All Ages
CD86	Elderly	Literature		Expressed in All Ages
CDC2	Elderly	Literature	Elderly	
CDC25C	Elderly	Literature		Expressed Sporadically
CDKN1A	Elderly	Literature		Expressed in All Ages
CDKN1B	Elderly	Literature		Expressed in All Ages
CDKN2C	Elderly	Literature		Expressed in All Ages
CFIX	Juvenile	Literature		No mRNA Detected
CGI-96	Juvenile	Affymetrix		Expressed in All Ages
CHR1orf28	Newborn	Affymetrix		Same Size mRNA/DNA
CIITA	Elderly	Literature		Expressed in All Ages
CLEC2	Elderly	Affymetrix		Expressed in All Ages
CLEC2a	Elderly	Affymetrix		Expressed in All Ages
CLEC2b	Elderly	Affymetrix		Same Size mRNA/DNA
COL1A1 CTx	Elderly	Literature		Expressed Sporadically
COL1A2	Newborn	Literature	Newborns	
COL6A1a	Juvenile	Affymetrix		Expressed Sporadically
COL6A1b	Juvenile	Affymetrix		No mRNA Detected
CTBP1	Elderly	Literature		Expressed in All Ages
CTSB	Elderly	Literature		Expressed in All Ages
CTSK	Elderly	Literature		Expressed in All Ages
CTSL	Elderly	Literature		Expressed Sporadically
CXorf22	Juvenile	Affymetrix		No mRNA Detected
CYP17A1	Juvenile	Literature		Expressed Sporadically
CYP1B1	Juvenile	Literature		No mRNA Detected
CYP7B1	Juvenile	Literature		Expressed Sporadically
CYTBC2	Elderly	Affymetrix		Same Size mRNA/DNA
DDB2	Elderly	Literature		Expressed in All Ages
DHEA	Juvenile	Literature		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
DNCL2A-1	Juvenile	Affymetrix		Expressed in All Ages
DNCL2A-2	Juvenile	Affymetrix		Expressed in All Ages
DNCL2A-3	Juvenile	Affymetrix		Expressed in All Ages
DNPEP	Juvenile	Affymetrix		Expressed in All Ages
DUSP6	Newborn	Affymetrix		Expressed in All Ages
DYRK2	Elderly	Literature		Expressed in All Ages
E2F1	Elderly	Literature		Expressed in All Ages
E2IG2	Juvenile	Literature		Expressed Sporadically
ECGF1	Elderly	Literature		No mRNA Detected
ELAVL1 HuR	Elderly	Literature		Expressed in All Ages
EMD	Elderly	Literature		Expressed in All Ages
ERBP	Juvenile	Literature		Expressed in All Ages
EREG	Elderly	Literature		No mRNA Detected
ERF	Elderly	Literature		Expressed in All Ages
ESR1	Juvenile	Literature		No mRNA Detected
ESR2	Juvenile	Literature		Expressed Sporadically
FACL6	Newborn	Affymetrix		No mRNA Detected
FKBP11	Juvenile	Affymetrix		Expressed in All Ages
FKLF	Newborn	Literature		Expressed in All Ages
FLJ11078	Juvenile	Affymetrix		Expressed in All Ages
FLJ20245	Elderly	Affymetrix		Same Size mRNA/DNA
FLJ20344a	Newborn	Affymetrix	Newborn	
FLJ20344b	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ20421	Elderly	Affymetrix		Same Size mRNA/DNA
FLJ21901	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ22175	Juvenile	Affymetrix		Expressed in All Ages
FLJ22672 PAQR6	Juvenile	Affymetrix		Expressed Sporadically
FLJ30658	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ35119	Juvenile	Affymetrix		Expressed in All Ages
FLJ35954	Newborn	Affymetrix		Same Size mRNA/DNA
FLJ35982	Juvenile	Affymetrix		Same Size mRNA/DNA
FLJ35982a	Juvenile	Affymetrix		No mRNA Detected
FLJ35984	Elderly	Affymetrix		Same Size mRNA/DNA
FLJ37440	Juvenile	Affymetrix		No mRNA Detected
FLJ38628	Elderly	Affymetrix		Expressed Sporadically
FLJ38745	Juvenile	Affymetrix		Same Size mRNA/DNA
FLJ43159	Juvenile	Affymetrix		Same Size mRNA/DNA
GADD45A	Elderly	Literature		Expressed in All Ages
GADD45B	Elderly	Literature		Expressed in All Ages
GAL	Juvenile	Literature		Expressed Sporadically

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
GFPT2	Juvenile	Affymetrix		Expressed Sporadically
GGT1	Juvenile	Affymetrix		Expressed in All Ages
GHRH	Juvenile	Literature		Same Size mRNA/DNA
GLO1	Juvenile	Literature		Expressed in All Ages
GNRH1	Juvenile	Literature		Expressed Sporadically
GNRH2	Juvenile	Literature		Same Size mRNA/DNA
GNRHR	Juvenile	Literature		No mRNA Detected
GPR54	Juvenile	Literature		Expressed Sporadically
GRIN1 NR1-1	Juvenile	Literature		Expressed Sporadically
GRIN1 NR1-2	Juvenile	Literature		No mRNA Detected
GRIN1 NR1-3	Juvenile	Literature		No mRNA Detected
GRIN2A	Juvenile	Literature		No mRNA Detected
GRIN2B	Juvenile	Literature		No mRNA Detected
GSTP1	Elderly	Affymetrix		Expressed in All Ages
H17	Juvenile	Affymetrix		Expressed in All Ages
HBA1	Adult	Literature		Expressed in All Ages
HBA2	Adult	Literature		Expressed in All Ages
HBB	Adult	Literature		Expressed in All Ages
HBD	Adult	Literature		Expressed in All Ages
HBE1	Newborn	Literature	Newborn	
HBG1	Newborn	Literature		Expressed in All Ages
HBG1n1	Newborn	Literature	Newborn	
HBG1n2	Newborn	Literature	Newborn	
HBG2	Newborn	Literature		Expressed in All Ages
HBG2n2	Newborn	Literature	Newborn	
HBG2n3	Newborn	Literature	Newborn	
HBZ	Newborn	Literature		No mRNA Detected
HBQ	Adult	Literature		Expressed Sporadically
HIC2	Elderly	Affymetrix		No mRNA Detected
HIF1A	Elderly	Literature		Expressed in All Ages
HOMER3	Juvenile	Affymetrix		Expressed Sporadically
HPCAL4	Elderly	Affymetrix		Expressed Sporadically
HRAS	Elderly	Literature		No mRNA Detected
HRG	Juvenile	Affymetrix		Expressed Sporadically
HTATIP	Elderly	Literature		Expressed in All Ages
HTR1E	Juvenile	Affymetrix		No mRNA Detected
HTR7	Juvenile	Affymetrix		Expressed Sporadically
IFNG	Elderly	Literature		Expressed Sporadically
IGF1	Elderly	Literature		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
IGF2	Elderly	Literature		No mRNA Detected
IGFBP3	Elderly	Literature	Elderly	
IGFBP5	Elderly	Literature		No mRNA Detected
IL1A	Elderly	Literature		Expressed Sporadically
INHA	Juvenile	Literature		No mRNA Detected
IRF1	Elderly	Literature		Expressed in All Ages
ITIH4	Juvenile	Affymetrix		Expressed Sporadically
ITSN2	Newborn	Affymetrix		Expressed in All Ages
KIAA0276	Newborn	Affymetrix		Expressed in All Ages
KIAA0894	Juvenile	Affymetrix		No mRNA Detected
KIAA1265	Newborn	Affymetrix		Expressed in All Ages
KIAA2022	Juvenile	Affymetrix		Expressed Sporadically
KISS-1	Juvenile	Literature		Expressed Sporadically
KITLG	Newborn	Literature		Expressed Sporadically
KL	Elderly	Literature		Expressed Sporadically
KLF13	Elderly	Literature		No mRNA Detected
LASS5	Elderly	Affymetrix		Expressed Sporadically
LATS1	Juvenile	Affymetrix		Expressed in All Ages
LEP	Juvenile	Literature		Expressed Sporadically
LHB	Juvenile	Literature		No mRNA Detected
LHCGR	Juvenile	Literature		No mRNA Detected
LMNA (norm)	Elderly	Literature		Expressed in All Ages
LMNA (RT)	Elderly	Literature		Expressed Sporadically
LMNA (spec)	Elderly	Literature		No mRNA Detected
LOC151194	Newborn	Affymetrix	Newborn	
LOC152274	Juvenile	Affymetrix		No mRNA Detected
LOC284242	Juvenile	Affymetrix		No mRNA Detected
LOH11CR2A	Elderly	Affymetrix	Elderly	
LZTFL1	Newborn	Affymetrix		Same Size mRNA/DNA
MAD1L1	Elderly	Literature	Elderly	
MCPH1	Elderly	Literature		Expressed in All Ages
MDM2	Elderly	Literature		Expressed in All Ages
MEPE	Elderly	Literature		No mRNA Detected
MET	Elderly	Literature		Expressed Sporadically
MGC14288	Elderly	Affymetrix		Expressed in All Ages
MGC20460	Juvenile	Affymetrix		Expressed in All Ages
MGC39650	Juvenile	Affymetrix		Expressed Sporadically
MIF	Newborn	Literature		No mRNA Detected
MLL	Elderly	Literature		No mRNA Detected
MMP-13	Elderly	Literature		No mRNA Detected

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
MMP-14	Elderly	Literature		Expressed Sporadically
MMP-9	Elderly	Literature		Expressed Sporadically
MS4A4A	Elderly	Affymetrix		Expressed Sporadically
MS4A4Aa	Elderly	Affymetrix		Expressed in All Ages
MS4A4Ab	Elderly	Affymetrix		Expressed Sporadically
MT1X	Elderly	Affymetrix		Expressed in All Ages
MYC	Elderly	Literature		Expressed in All Ages
NALP14	Juvenile	Affymetrix		Expressed Sporadically
NBN	Elderly	Literature		No mRNA Detected
NDE1	Juvenile	Affymetrix		Expressed in All Ages
NMI	Elderly	Literature		Expressed in All Ages
NPPB	Elderly	Literature		No mRNA Detected
NRAS	Elderly	Literature		Expressed in All Ages
NTS	Juvenile	Literature		No mRNA Detected
OGG1	Elderly	Literature		Expressed Sporadically
OPG TNFRSF11B	Elderly	Literature		No mRNA Detected
OPGL RANKL TNFSF11	Elderly	Literature		Expressed Sporadically
OSGEP	Juvenile	Affymetrix		Expressed in All Ages
OSM	Elderly	Literature		Expressed Sporadically
OXTR	Juvenile	Literature		Expressed Sporadically
PAQR6	Juvenile	Affymetrix		No mRNA Detected
PDCD1	Elderly	Literature		Expressed Sporadically
PDCD10 CCM3	Elderly	Literature		Expressed in All Ages
PDCD11	Elderly	Literature		Expressed in All Ages
PDCD1LG2	Elderly	Literature		Expressed Sporadically
PDCD2L	Elderly	Literature		Expressed Sporadically
PDCD4	Elderly	Literature		Expressed in All Ages
PDCD5	Elderly	Literature		Expressed in All Ages
PDCD6	Elderly	Literature	Elderly	
PDCD6IP	Elderly	Literature		Expressed in All Ages
PDCD7	Elderly	Literature		Expressed in All Ages
PDE6D	Juvenile	Affymetrix		Expressed in All Ages
PGR	Juvenile	Literature		No mRNA Detected
PIK3CA	Elderly	Literature		Expressed in All Ages
PITPNC1	Newborn	Affymetrix		Same Size mRNA/DNA
PLEKHA8	Juvenile	Affymetrix		Same Size mRNA/DNA

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
POLA1	Elderly	Literature		Expressed in All Ages
POLA2	Elderly	Literature		Expressed in All Ages
POLB	Elderly	Literature		Expressed in All Ages
POLD1	Elderly	Literature		Expressed in All Ages
POLE1	Elderly	Literature		Expressed in All Ages
POLE2	Elderly	Literature		Expressed in All Ages
POLE3	Elderly	Literature		Expressed in All Ages
POLG	Elderly	Literature		Expressed in All Ages
POLH	Elderly	Literature		Expressed in All Ages
POLI	Elderly	Literature		Expressed in All Ages
POLK	Elderly	Literature		Expressed in All Ages
POLM	Elderly	Literature	Elderly	
POLN	Elderly	Literature		No mRNA Detected
POLQ	Elderly	Literature	Elderly	
POLR3F	Elderly	Literature		Expressed in All Ages
POLR3K	Elderly	Literature		Expressed in All Ages
POLS	Elderly	Literature		Expressed in All Ages
POMC	Juvenile	Literature		No mRNA Detected
POT1	Elderly	Literature		Expressed in All Ages
PPARD	Elderly	Literature	Elderly	
PPAT	Newborn	Affymetrix		Same Size mRNA/DNA
PPOX	Juvenile	Literature	Juvenile	
PRDX5	Elderly	Literature		Expressed in All Ages
PRKCA	Elderly	Literature		Expressed in All Ages
PRL	Juvenile	Literature	Juvenile	
PTGER4	Elderly	Literature		Expressed in All Ages
PTH	Juvenile	Literature		No mRNA Detected
PTMS	Juvenile	Affymetrix		Same Size mRNA/DNA
PTPN18	Elderly	Affymetrix		Expressed Sporadically
RAD50	Elderly	Literature		Expressed in All Ages
RAF1	Elderly	Literature		No mRNA Detected
RaI	Newborn	Affymetrix		Same Size mRNA/DNA
RaIGPS2	Newborn	Affymetrix		Expressed in All Ages
RANK	Elderly	Literature		Expressed Sporadically
RAPA-2 TRERF1	Juvenile	Literature		Expressed in All Ages
RARA	Juvenile	Affymetrix		Expressed in All Ages
RB1	Elderly	Literature		Expressed in All Ages
RBL1	Elderly	Literature		Expressed in All Ages
RBL2	Elderly	Literature		Expressed in All Ages

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
REA	Juvenile	Literature		Same Size mRNA/DNA
RELA	Elderly	Literature		Expressed in All Ages
RUNX2	Elderly	Literature		Expressed in All Ages
SEMA4A	Elderly	Affymetrix		Expressed in All Ages
SH3GL1	Juvenile	Affymetrix		Expressed in All Ages
SHBG	Juvenile	Literature		No mRNA Detected
SLC20A1	Elderly	Literature		Expressed in All Ages
SLC39A4	Juvenile	Affymetrix		Same Size mRNA/DNA
SMG5	Elderly	Literature		Expressed in All Ages
SMG6	Elderly	Literature		Expressed in All Ages
SMG7	Elderly	Literature		Expressed in All Ages
SNCA	Elderly	Literature		Expressed in All Ages
SPATA1 SP2	Juvenile	Literature		Expressed Sporadically
SPINK5L3	Juvenile	Affymetrix		Same Size mRNA/DNA
SPINKa	Juvenile	Affymetrix		Same Size mRNA/DNA
SPINKb	Juvenile	Affymetrix		No mRNA Detected
SPP1	Elderly	Literature		Expressed Sporadically
SPTRX-1	Juvenile	Literature	Juvenile	
SPTRX-2	Juvenile	Literature	Juvenile	
SRC	Elderly	Literature	Elderly	
SRPX	Juvenile	Affymetrix		No mRNA Detected
SST	Juvenile	Literature		No mRNA Detected
STAF42	Newborn	Affymetrix		Same Size mRNA/DNA
STK16	Elderly	Affymetrix		Expressed Sporadically
TBC1	Juvenile	Affymetrix	Juvenile	
TEKT2	Juvenile	Literature	Juvenile	
TEP1	Elderly	Literature		No mRNA Detected
TERF2	Elderly	Literature		No mRNA Detected
TERT	Elderly	Literature		No mRNA Detected
TFAP2BL1	Juvenile	Affymetrix		No mRNA Detected
TINF2	Elderly	Literature		Expressed in All Ages
TNFAIP3	Elderly	Literature		Expressed in All Ages
TNFRSF11A	Elderly	Literature		Expressed Sporadically
TNFSF10	Elderly	Literature		Expressed in All Ages
TNIP1	Elderly	Literature		Expressed in All Ages
TNKS1BP1	Elderly	Literature		Expressed Sporadically

Candidate Gene	Target Age Group	Gene Origin	Accepted Candidates	Rejected Candidates
TP53	Elderly	Literature		Expressed in All Ages
TP53BP1	Elderly	Literature		No mRNA Detected
TP53BP2	Elderly	Literature		No mRNA Detected
TP53I3	Elderly	Literature		No mRNA Detected
TP73	Elderly	Literature		No mRNA Detected
TPST1	Elderly	Affymetrix		Expressed Sporadically
TRPC1	Juvenile	Affymetrix		Expressed Sporadically
TSLL2	Juvenile	Affymetrix		Expressed in All Ages
TUFT1	Juvenile	Affymetrix		Expressed in All Ages
UNQ501	Elderly	Affymetrix		Expressed in All Ages
VSIG2	Elderly	Literature		Expressed Sporadically
WHSC1L1	Newborn	Affymetrix		Same Size mRNA/DNA
WRN	Elderly	Literature		Expressed Sporadically
XTP3TPA	Elderly	Affymetrix		Expressed Sporadically

APPENDIX G: CANDIDATE GENE PRIMER SEQUENCES FOR qRT-PCR

Table 28: Candidate Gene Primer Sequences for Real-Time RT-PCR.

Candidate Gene	Target Age Group	Primer and MGB Probe Sequences 5' → 3'	NCBI Genbank Accession Number
AGGF1	Elderly	502F aagctgctgcatcacacagaac 568T 6FAMcaggtggaagaacMGBNFQ 604R tcccacgttggagtattttactga	NM_018046
ASL	Juvenile	594F ctgcagtgacagctggttgag 676T 6FAMaatggcatccctttgcMGBNFQ 727R acatgctggccactgacctt	NM_001024943
CDC2	Elderly	881F acctggaatcctgcataagca 904T 6FAMtcctgaagactgactatatMGBNFQ 948R tctattaaaggaacttcgtcatccaa	NM_001786
COL1A2	Newborn	1383F gcatccttggttagggtcaatc 1406T 6FAMagtagtaaccactgctccMGBNFQ 1456R catgccgtgacttgagactca	NM_000089
FLJ20344a	Newborn	318F gcgaagcctgatgtgatcttc 395T 6FAMctgtgcagaagtctggMGBNFQ 455R tttgtcttggctttccttgtagtg	NM_017776
HBE1	Newborn	671F attgccctggcccataagta 697T 6FAMagttctcttccagtttgcagMGBNFQ 743R aggagggtgtcagggtcaca	NM_005330
HBG1n1	Newborn	61F gaaagctctgaatcatccaggtg 85T 6FAMtttgtggcatctcccaaggaagtcagcMGBNFQ 134R agtcaaggcacatggcaagaag	N/A
HBG2n3	Newborn	78F gcagtgagctcagtgcagttc 110T 6FAMcaaaggtgcccttgagatcatccaggMGBNFQ 159R ttccttgggagatgccataaa	N/A
IGFBP3	Elderly	743F agaacttctcctccgagtccaa 772T 6FAMacagaatatggtccctgccMGBNFQ 822R caggtgattcagtgtgtcttcca	NM_001013398
LOC151194	Newborn	400F gggctggtgggcatagtg 423T 6FAMcctgctgggtgctcaMGBNFQ 461R acttttcgatccgtgatagtcaca	NM_145280
LOH11CR2A	Elderly	1178F cttggcaccactccagaaca 1227T 6FAMcccctacagcttttMGBNFQ 1277R actaaacgtgtctgtaacttctccatct	BC001234
MAD1L1	Elderly	696F caggcagtgtcagcagaacttg 767T 6FAMctggcgagaccatcaaMGBNFQ 805R ccgagatcctccccttcagt	NM_003550

Candidate Gene	Target Age Group	Primer and MGB Probe Sequences 5' → 3'	NCBI Genbank Accession Number
PDCD6	Elderly	429F tcgataagaacgagctgaagca 459T 6FAMcaggtttcggctaccgMGBNFQ 498R atgtcgtggaactggtcagaga	NM_013232
POLM	Elderly	1095F ggcccaggtgtctgaagatg 1140T 6FAMatgtcttctgctccgggtMGBNFQ 1176R aacagccatgggctgtttg	NM_013284
POLQ	Elderly	6374F cagcccagacggttggaa 6403T 6FAMcatccctgcataaacMGBNFQ 6459R tgcaatcgtgggcagattc	NM_199420
PPARD	Elderly	906F catcctcaccggcaaagc 929T 6FAMacacggcgcccttMGBNFQ 963R tgtctcgatgtcgtggatcac	NM_006238
PPOX	Juvenile	791F aaacccatcgttccatattactgg 826T 6FAMtccgccctgccccMGBNFQ 869R tggcgaatgagtgctgagtc	NM_000309
PRL	Juvenile	983F ttctagagggcatggagctgat 1007T 6FAMtcagccaggttcatcMGBNFQ 1049R gggtagatctcattttctttggtttc	NM_000948
SPTRX-1	Juvenile	244F gagggaaaaccaactgtaacgtg 268T 6FAMcacccaaataaagctcaMGBNFQ 308R cacgttgctggacaggactagt	NM_032243
SPTRX-2	Juvenile	531F aaatgaactgaacgaagacgaaatt 564T 6FAMtgctgtcgcagaagcMGBNFQ 607R taaatggctgcaaagtcacaatg	NM_016616
SRC	Elderly	893F tgaggagtggtattttggcaaga 995T 6FAMcacgaaaggtgcctactMGBNFQ 1040R ggcgttgtcgaagtcagaca	NM_005417
TBC1	Juvenile	729F gctatgtgttcaaagccgatga 752T 6FAMcaaacaaaatgctcatcatcMGBNFQ 803R ctccggcagctctttcaaag	BC028196
TEKT2	Juvenile	700F tctcaacctcagatccccaaa 752T 6FAMcctgatggctccaccaMGBNFQ 808R gtccttgttgaaccgactgaagt	NM_014466

Candidate Gene	Target Age Group	Primer and MGB Probe Sequences 5' → 3'	NCBI Genbank Accession Number
GNAS	All Ages	1653F ggacaaagtcaacttccacatgttt 1690T NEDcagcgcgatgaacgccgcaaMGBNFQ 1749R gaagatgatggcagtcacatcgt	NM_016592
S15	All Ages	16F ccaaagcgatctcttctgaggat 40T VICcggcaagatggcagaagtagagcagaaMGBNFQ 105R acgccgcggtaggtgaa	NM_001018

APPENDIX H: UCF IRB FORM

UNIVERSITY OF CENTRAL FLORIDA

RESEARCH OR CLASSROOM USE INFORMED CONSENT FORM

Dr. Jack Ballantyne **Department of Chemistry**

I. **PROJECT TITLE:** The Determination of the Physical Characteristics of an Individual From Biological Stains

II. **INTRODUCTION:** The ability to determine the physical characteristics of an individual depositing a particular biological stain at a crime scene by investigating potentially useful human physical biometric indicators that may be amenable to molecular genetic analysis at this time, including age, skin pigmentation and ethnogeographic ancestry. The proposed work seeks to investigate whether determination of an individual's age is feasible in dried physiological stains. The possibility of identifying a number of potential RNA 'molecular clocks' that could provide investigators with information as to whether a DNA donor is a newborn, infant, toddler, child, adolescent, adult, middle-aged individual or old-aged individual. The strategy will be to identify genes that are differentially expressed (at the RNA level) during the various phases of human development.

III. I have volunteered to take part in this study. I understand that my participation is not required. If I am a student, refusal to participate in this study will not affect my grade in any way. I understand that participation in this study does not require that I donate all sample types, only the ones I am comfortable giving. My physiological sample can be used for the following purpose(s):

____ 1. To provide samples for use in a classroom setting to teach students the techniques used in the analysis of forensic evidence, or for use on ongoing projects in the research lab. The students and/or researchers will perform the routine tests used in a forensic laboratory or conduct original research using these samples. The students in the laboratory will observe universal precautions at all times when handling the samples (i.e. gloves, lab coats, appropriate clothing, safety glasses when appropriate, etc.) to protect themselves from contamination by any infectious agents that may be present in the samples.

____ 2. To be dried and preserved to be used in a teaching lab, as part of research, and any population studies undertaken.

____ 3. To be counted in the population study or for the advancement of research projects.

____ 4. I am a volunteer who knows about this research through word of mouth and wish to submit my sample on the basis that I will not be able to obtain the results of my sample.

APPROVED BY
University of Central Florida
Institutional Review Board
9/8/06
CHAIRMAN

Note: Please initial and date Items 3 or 4, then choose Items 1 or 2 or both, if you wish to participate in this study.

IV. The genetic analysis of human physiological samples will take place in the University of Central Florida Chemistry Department, Laboratory 305 or at 12354 Research Parkway, Laboratory 321/322 in Orlando, Florida.

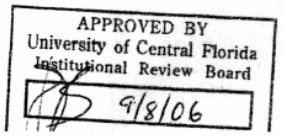
V. Participation requires a one-time donation, with no further donations required. The amount of time necessary for this donation will vary depending on its nature. Participants will not receive compensation for their donations. This is what will happen to the donor:

1. Liquid blood donations- the donor will go to the University of Central Florida MLS (medical laboratory sciences) laboratory, where a one-time donation of 10 – 15 mL of blood will be drawn by qualified personnel (**Jeneatte Vance**). Donation will take approximately 5 minutes. Subjects will be healthy, non-pregnant adults who weigh at least 110 pounds. The blood will be contained in standard, purple-topped Vacutainers. The personnel drawing the blood will use standard sterile techniques. These include the use of sterile, one-time use needles, disposable gloves (to be discarded after each sample is drawn) and sterile blood collection tubes. The subject's arm will be swabbed with alcohol before the needle is inserted. The site of the puncture will be bandaged after the blood has been drawn.

2. Other physiological donations, such as semen, saliva, and vaginal secretions, will be deposited on stain cards, dried on swabs, or submitted in a sealed container. Upon receipt, laboratory personnel will handle the samples using universal precautions. Semen swabs are collected by the donor in his home as follows: the subject ejaculates into a plastic waste cup (provided to him). He then places approximately 15 sterile swabs upside down in the cup. The swabs soak up the fluid and are allowed to dry overnight, and are returned to researchers. Alternatively, if liquid semen is required, the plastic cup will be simply sealed after collection and returned to researchers. Vaginal swabs are collected by the donor in her home. She swabs the cervical region with sterile swabs, allows them to dry overnight and returns them to researchers.

3. Each sample will be coded with a unique number. This number, the subject's gender, and the population group to which he/she belongs will be the only individual characteristics known to research personnel and/or students. Under no circumstances will a sample be associated with a name, and a donor will not be able to obtain any information about his/her sample.

4. My physiological sample will be dried on to cards or swabs and frozen for long-term storage purposes. The samples may be used in a classroom setting to allow students to learn forensic techniques. The samples may also be used in original research projects and a compilation of genetic databases consisting of all individuals and their genotypes for every marker used in the lab.



5. I will consent to the use of my sample as described by initialing and dating section III of this form.

VI. Sometimes there are side effects after blood is drawn with a needle. These side effects are called risks, and for this project, the risks are:

1. Mild discomfort when my blood is removed by needle.
2. Possible bruising at the site of the needle puncture. This possibility is reduced by holding your arm above your head just after the procedure.
3. Possible weakness, fainting or dizziness which may be reduced by sitting down for several minutes after the blood is drawn.
4. Possible contamination at the site where the blood was drawn which will be reduced by the application of a bandage.

VII. There are no direct benefits to the participants, but indirect benefits would include the improvement of the forensic program and the development or discovery of molecular markers of the age of a biological stain.

VIII. My confidentiality will be guarded. University of Central Florida will protect all the information about me and my part in this study as required by the IRB and privacy laws for research participants just as is done for all subjects at University of Central Florida.

IX. If you believe you have been injured during participation in this research project, you may file a claim with UCF Environmental Health & Safety, Risk and Insurance Office, P.O. Box 163500, Orlando, FL 32816-3500 (407) 823-6300. The University of Central Florida is an agency of the State of Florida for purposes of sovereign immunity and the university's and the state's liability for personal injury or property damage is extremely limited under Florida law. Accordingly, the university's and the state's ability to compensate you for any personal injury or property damage suffered during this research project is very limited.
Information regarding your rights as a research volunteer may be obtained from:

Barbara Ward
Institutional Review Board (IRB)
University of Central Florida (UCF)
12201 Research Parkway, Suite 501
Orlando, FL 32826-3246
Telephone: (407) 823-2901

X. I understand my physiological sample will become an anonymous sample identified only by a number. Any genetic data obtained from this sample will only be used for counting purposes in the population study, or for the advancement of research projects. The genetic data developed in the classroom will be used for sample to sample comparison for learning purposes. I will not be able, under any circumstances, to obtain any information about my sample.

APPROVED BY
University of Central Florida
Institutional Review Board
9/8/06

I understand I may feel pain when my blood is removed by needle and may receive bruising at this site. I also understand I may experience weakness, fainting or dizziness as and will be required to sit down for several minutes after the blood is drawn to minimize these adverse effects.

Participation in this study is my choice. If I do not take part, there will be no penalty. Also, if I do agree to take part, I may stop at any point without penalty.

XI. Signature: _____ Date:_____

If you have any questions, you may contact the principal investigator:

Jack Ballantyne, PhD
Associate Professor, Department of Chemistry
Associate Director for Research,
National Center for Forensic Science
University of Central Florida
PO Box 162366
Orlando, FL 32816-2366
Phone: 407-823-0163
Fax: 407-823-2252

Or the Lab Manager:

Mindy Setzer
National Center for Forensic Science
PO Box 162367
Orlando, FL 32826-2367
Phone: 407-823-4041
Fax: 407-823-4042

XII. If you will be taking part in the population frequency study, please indicate your population group by marking a choice below:

Sex: Male	_____
Female	_____
Caucasian	_____
African American	_____
Asian	_____
(South Eastern U.S.) Hispanic	_____
(South Western U.S.) Hispanic	_____
American Indian	_____

Other (please identify): _____

APPROVED BY
University of Central Florida
Institutional Review Board
9/8/06
CHAIRMAN

I have read and understand the information in this form. I am at least 18 years of age, my questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research entitled "The Determination of the Physical Characteristics of and Individual From Biological Stains."

Printed name:_____

Signature:_____

Date:_____

Relationship if not self:_____

Witness:_____

Witness:_____

Relationship if not self:_____

APPROVED BY
University of Central Florida
Institutional Review Board
9/8/06
CHAIRMAN

5

Figure 48: UCF IRB Form.

LIST OF REFERENCES

1. Budowle, B., et al. CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools. in Proceedings of the Second European Symposium on Human Identification. 1998. Madison, Wisconsin: Promega Corporation (http://www.promega.com/geneticidproc/eusymp2proc/17.pdf).

2. Moretti, T.R., et al., Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. J. Forensic Sci., 2001. 46(3): p. 647-660.

3. Lamason, R.L., et al., SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science, 2005. 310(5755): p. 1782-1786.

4. Rees, J.L., Genetics of hair and skin color. Annu. Rev. Genet., 2003. 37: p. 67-90.

5. Frudakis, T., et al., Sequences associated with human iris pigmentation. Genetics, 2003. 165(4): p. 2071-2083.

6. Hirschhorn, J.N., Genetic and genomic approaches to studying stature and pubertal timing. Pediatr. Endocrinol. Rev., 2005. 2 Suppl 3: p. 351-354.

7. Francis-West, P.H., L. Robson, and D.J. Evans, Craniofacial development: the tissue and molecular interactions that control development of the head. Adv. Anat. Embryol. Cell Biol., 2003. 169: p. III-VI, 1-138.

8. Grimes, E.A., et al., Sequence polymorphism in the human melanocortin 1 receptor gene as an indicator of the red hair phenotype. Forensic Sci. Int., 2001. 122(2-3): p. 124-129.

9. Seifert, K.L. and R. Hoffnung, Child and Adolescent Development. 5th ed. 2000, Boston: Houghton Mifflin.

10. Meissner, C., N. von Wurmb, and M. Oehmichen, Detection of the age-dependent 4977 bp deletion of mitochondrial DNA. A pilot study. Int. J. Legal Med., 1997. 110(5): p. 288-291.

11. Michikawa, Y., et al., Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. Science, 1999. 286(5440): p. 774-779.

12. Cortopassi, G.A., et al., A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues. Proc. Natl. Acad. Sci. USA, 1992. 89(16): p. 7370-7374.

13. Liu, V.W., C. Zhang, and P. Nagley, Mutations in mitochondrial DNA accumulate differentially in three different human tissues during ageing. Nucleic Acids Res., 1998. 26(5): p. 1268-1275.

14. Tsuji, A., et al., Estimating age of humans based on telomere shortening. Forensic Sci. Int., 2002. 126(3): p. 197-199.

15. Figueroa, R., et al., Telomere erosion varies during in vitro aging of normal human fibroblasts from young and adult donors. Cancer Res., 2000. 60(11): p. 2770-2774.

16. Baynes, J.W., The role of AGEs in aging: causation or correlation. Exp. Gerontol., 2001. 36(9): p. 1527-1537.

17. Lezza, A.M., et al., Correlation between mitochondrial DNA 4977-bp deletion and respiratory chain enzyme activities in aging human skeletal muscles. Biochem. Biophys. Res. Commun., 1994. 205(1): p. 772-779.

18. Lenaz, G., et al., Role of mitochondria in oxidative stress and aging. Ann. N. Y. Acad. Sci., 2002. 959: p. 199-213.

19. Hamilton, M.L., et al., Does oxidative damage to DNA increase with age? Proc. Natl. Acad. Sci. USA, 2001. 98(18): p. 10469-10474.

20. Beckman, K.B. and B.N. Ames, The free radical theory of aging matures. Physiol. Rev., 1998. 78(2): p. 547-581.

21. International, et al., Finishing the euchromatic sequence of the human genome. Nature, 2004. 431(7011): p. 931-945.

22. Alvarez, M. and J. Ballantyne, The identification of newborns using messenger RNA profiling analysis. Anal Biochem, 2006. 357(1): p. 21-34.

23. Lee, C.K., et al., Gene expression profile of aging and its retardation by caloric restriction. Science, 1999. 285(5432): p. 1390-1393.

24. Touchberry, C.D., et al., Age-related changes in relative expression of real-time PCR housekeeping genes in human skeletal muscle. J Biomol Tech, 2006. 17(2): p. 157-162.

25. Dozmorov, I., A. Bartke, and R.A. Miller, Array-based expression analysis of mouse liver genes: effect of age and of the longevity mutant Prop1df. J. Gerontol. A. Biol. Sci. Med. Sci., 2001. 56(2): p. B72-80.

26. Lee, C.K., R. Weindruch, and T.A. Prolla, Gene-expression profile of the ageing brain in mice. Nat. Genet., 2000. 25(3): p. 294-297.

27. Olze, A., et al., Forensic age estimation in living subjects: the ethnic factor in wisdom tooth mineralization. Int J Legal Med, 2004. 118(3): p. 170-173.

28. Takasaki, T., et al., Age estimation in dental pulp DNA based on human telomere shortening. Int J Legal Med, 2003. 117(4): p. 232-234.

29. Ly, D.H., et al., Mitotic misregulation and human aging. Science, 2000. 287(5462): p. 2486-2492.

30. Jonsson, M., et al., Hash4, a novel human achaete-scute homologue found in fetal skin. Genomics, 2004. 84(5): p. 859-866.

31. Jane, S.M. and J.M. Cunningham, Molecular mechanisms of hemoglobin switching. Int. J. Biochem. Cell Biol., 1996. 28(11): p. 1197-1209.

32. Stamatoyannopoulos, G., Control of globin gene expression during development and erythroid differentiation. Exp Hematol, 2005. 33(3): p. 259-271.

33. Martin, D.I., S. Fiering, and M. Groudine, Regulation of beta-globin gene expression: straightening out the locus. Curr. Opin. Genet. Dev., 1996. 6(4): p. 488-495.

34. Bernards, R., et al., Structure of the human G gamma-A gamma-delta-beta-globin gene locus. Proc Natl Acad Sci U S A, 1979. 76(10): p. 4827-4831.

35. Brittain, T., Molecular aspects of embryonic hemoglobin function. Mol Aspects Med, 2002. 23(4): p. 293-342.

36. PreAnalytiX, PAXgeneTM Blood RNA Kit Handbook. 2001.

37. AFFYMETRIX, Technical Note: Globin Reduction Protocol: A Method for Processing Whole Blood RNA Samples for Improved Array Results. 2003.

38. AFFYMETRIX, Technical Note: An Analysis of Blood Processing Methods to Prepare Samples for GeneChip Expression Profiling. 2003.

39. AFFYMETRIX, Data Sheet: GeneChip Human Genome Arrays. 2003.

40. Expression and Analysis, Definitions and Illustrations of Data from Expression Analysis Using AFFYMETRIX GeneChips. 2003.

41. AFFYMETRIX, Technical Manual: GENE EXPRESSION MONITORING GeneChip Expression Analysis. 1999.

42. AFFYMETRIX, Statistical Alogrithms Reference Guide. 2001.

43. AFFYMETRIX, Appendix 5: GeneChip 3.1 Expression Analysis Alogorithm Tutorial. 2001.

44. Chomczynski, P. and N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., 1987. 162(1): p. 156-159.

45. Huang, Z., M.J. Fasco, and L.S. Kaminsky, Optimization of Dnase I removal of contaminating DNA from RNA for use in quantitative RNA-PCR. Biotechniques, 1996. 20(6): p. 1012-1014, 1016, 1018-1020.

46. Wiame, I., et al., Irreversible heat inactivation of DNase I without RNA degradation. Biotechniques, 2000. 29(2): p. 252-254, 256.

47. Jones, L.J., et al., RNA quantitation by fluorescence-based solution assay: RiboGreen reagent characterization. Anal Biochem, 1998. 265(2): p. 368-374.

48. Ambion, RETROscriptTM First-Strand Synthesis Kit for RT-PCR Instruction Manual. 1997.

49. Gerard, G.F., et al., Reverse transcriptase. The use of cloned Moloney murine leukemia virus reverse transcriptase to synthesize DNA from RNA. Mol Biotechnol, 1997. 8(1): p. 61-77.

50. Shiga, K., H. Yamamoto, and H. Okamoto, Isolation and characterization of the human homologue of rig and its pseudogenes: the functional gene has features characteristic of housekeeping genes. Proc Natl Acad Sci U S A, 1990. 87(9): p. 3594-3598.

51. Kitagawa, M., et al., rig encodes ribosomal protein S15. The primary structure of mammalian ribosomal protein S15. FEBS Lett, 1991. 283(2): p. 210-214.

52. Livak, K.J. and T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 2001. 25(4): p. 402-408.

53. Alvarez, M., J. Juusola, and J. Ballantyne, An mRNA and DNA co-isolation method for forensic casework samples. Anal Biochem, 2004. 335(2): p. 289-298.

54. Comey, C.T., et al., DNA extraction strategies for amplified fragment length polymorphism analysis. J. Forensic Sci., 1994. 39: p. 1254–1269.

55. Green, R.L., et al., Developmental validation of the quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. J Forensic Sci, 2005. 50(4): p. 809-825.

56. Cawthon, R.M., Telomere measurement by quantitative PCR. Nucleic Acids Res, 2002. 30(10): p. e47.

57. Baird, D.M., et al., Extensive allelic variation and ultrashort telomeres in senescent human cells. Nat Genet, 2003. 33(2): p. 203-207.

58. Expression and Analysis, Technical Note: Two-Group Comparison, Multiple Samples. 2003.

59. Wang, W., et al., Loss of HuR is linked to reduced expression of proliferative genes during replicative senescence. Mol Cell Biol, 2001. 21(17): p. 5889-5898.

60. Brewer, G., Messenger RNA decay during aging and development. Ageing Res Rev, 2002. 1(4): p. 607-625.

61. Asano, H., X.S. Li, and G. Stamatoyannopoulos, FKLF, a novel Kruppel-like factor that activates human embryonic and fetal beta-like globin genes. Mol Cell Biol, 1999. 19(5): p. 3571-3579.

62. Garces, C., et al., Effects of dehydroepiandrosterone-sulfate on the Apo E genotype influence on plasma lipid levels in prepubertal children. J Clin Endocrinol Metab, 2003. 88(8): p. 3997-4000.

63. Marodi, L., Innate cellular immune responses in newborns. Clin Immunol, 2006. 118(2-3): p. 137-144.

64. Lee, P.R., D. Brady, and J.I. Koenig, Corticosterone alters N-methyl-D-aspartate receptor subunit mRNA expression before puberty. Brain Res Mol Brain Res, 2003. 115(1): p. 55-62.

65. Tsuchiya, Y., et al., Human CYP1B1 is regulated by estradiol via estrogen receptor. Cancer Res, 2004. 64(9): p. 3119-3125.

66. Richardson, H.N., et al., Increased expression of forebrain GnRH mRNA and changes in testosterone negative feedback following pubertal maturation. Mol Cell Endocrinol, 2004. 214(1-2): p. 63-70.

67. Rossmanith, W.G., et al., Induction of galanin gene expression in gonadotropin-releasing hormone neurons with puberty in the rat. Endocrinology, 1994. 135(4): p. 1401-1408.

68. Schoof, E., et al., Comparison of leptin gene expression in different adipose tissues in children and adults. Eur J Endocrinol, 2004. 150(4): p. 579-584.

69. Vogel, G., Reproductive biology. A powerful first KiSS-1. Science, 2005. 309(5734): p. 551-552.

70. Urbanski, H.F., Leptin and puberty. Trends Endocrinol Metab, 2001. 12(10): p. 428-429.

71. Bello, A.R., et al., Developmental expression of neurotensin in thyrotropes and gonadotropes of male and female rats. Neuroendocrinology, 2004. 79(2): p. 90-99.

72. Wiemann, J.N., D.K. Clifton, and R.A. Steiner, Pubertal changes in gonadotropin-releasing hormone and proopiomelanocortin gene expression in the brain of the male rat. Endocrinology, 1989. 124(4): p. 1760-1767.

73. Kerrigan, J.R., et al., Augmented hypothalamic proopiomelanocortin gene expression with pubertal development in the male rat: evidence for an androgen receptor-independent action. Endocrinology, 1991. 128(2): p. 1029-1035.

74. Chowen, J.A., et al., Effects of the neonatal sex steroid environment on growth hormone-releasing hormone and somatostatin gene expression. J Pediatr Endocrinol, 1993. 6(3-4): p. 211-218.

75. Pugeat, M., et al., Clinical utility of sex hormone-binding globulin measurement. Horm Res, 1996. 45(3-5): p. 148-155.

76. Janne, M., et al., Expression and regulation of human sex hormone-binding globulin transgenes in mice during development. Endocrinology, 1999. 140(9): p. 4166-4174.

77. Argente, J. and J.A. Chowen, Control of the transcription of the growth hormone-releasing hormone and somatostatin genes by sex steroids. Horm Res, 1993. 40(1-3): p. 48-53.

78. Chowen, J.A., et al., Differential effects of the neonatal and adult sex steroid environments on the organization and activation of hypothalamic growth hormone-releasing hormone and somatostatin neurons. Endocrinology, 1993. 133(6): p. 2792-2802.

79. Burkle, A., S. Beneke, and M.L. Muiras, Poly(ADP-ribosyl)ation and aging. Exp Gerontol, 2004. 39(11-12): p. 1599-1601.

80. Comporti, M., et al., Plasma F2-isoprostanes are elevated in newborns and inversely correlated to gestational age. Free Radic Biol Med, 2004. 37(5): p. 724-732.

81. Reix, S., et al., Expression of cortical and hippocampal apoptosis-inducing factor (AIF) in aging and Alzheimer's disease. Neurobiol Aging, 2007. 28(3): p. 351-356.

82. Scaffidi, P. and T. Misteli, Lamin A-dependent nuclear defects in human aging. Science, 2006. 312(5776): p. 1059-1063.

83. Zhang, Y., et al., Caspase-2 deficiency enhances aging-related traits in mice. Mech Ageing Dev, 2007. 128(2): p. 213-221.

84. Petit, N., et al., Patterns of expression of the three cerebral cavernous malformation (CCM) genes during embryonic and postnatal brain development. Gene Expr Patterns, 2006. 6(5): p. 495-503.

85. Kerschan-Schindl, K., et al., Serum levels of cathepsin K decrease with age in both women and men. Exp Gerontol, 2005. 40(6): p. 532-535.

86. Goldstein, S., E.J. Moerman, and R.C. Baxter, Accumulation of insulin-like growth factor binding protein-3 in conditioned medium of human fibroblasts increases with chronologic age of donor and senescence in vitro. J Cell Physiol, 1993. 156(2): p. 294-302.

87. Grigoriev, V.G., E.J. Moerman, and S. Goldstein, Senescence and cell density of human diploid fibroblasts influence metabolism of insulin-like growth factor binding proteins. J Cell Physiol, 1994. 160(1): p. 203-211.

88. Moerman, E.J., et al., Insulin-like growth factor binding protein-3 is overexpressed in senescent and quiescent human fibroblasts. Exp Gerontol, 1993. 28(4-5): p. 361-370.

89. Frank, M.G., et al., mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. Neurobiol Aging, 2006. 27(5): p. 717-722.

90. Hamet, P. and J. Tremblay, Genes of aging. Metabolism, 2003. 52(10 Suppl 2): p. 5-9.

91. Taira, N., et al., DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. Mol Cell, 2007. 25(5): p. 725-738.

92. Campisi, J., et al., Cellular senescence, cancer and aging: the telomere connection. Exp. Gerontol., 2001. 36(10): p. 1619-1637.

93. Boland, E.J., et al., Age-specific regulation of clotting factor IX gene expression in normal and transgenic mice. Blood, 1995. 86(6): p. 2198-2205.

94. Huehns, E.R., The structure and function of haemoglobin: clinical disorders due to abnormal hemoglobin structure. Blood and its Disorders, ed. R.M. Hardiston and D.J. Weatherall. 1974, Oxford, UK.: Blackwell Scientific.

95. Cooper, H.A. and H.C. Hoagland, Fetal hemoglobin. Mayo Clin Proc, 1972. 47(6): p. 402-414.

96. Rochette, J., J.E. Craig, and S.L. Thein, Fetal hemoglobin levels in adults. Blood Rev, 1994. 8(4): p. 213-224.

97. Huisman, T.H., The structure and function of normal and abnormal haemoglobins. Baillieres Clin Haematol, 1993. 6(1): p. 1-30.

98. Perez-Stable, C., et al., Prostate, adrenocortical, and brown adipose tumors in fetal globin/T antigen transgenic mice. Lab Invest, 1996. 74(2): p. 363-373.

99. Lutay, A.V., M.A. Zenkova, and V.V. Vlassov, Nonenzymatic Recombination of RNA: Possible Mechanism for the Formation of Novel Sequences. Chem Biodivers, 2007. 4(4): p. 762-767.

100. Bekaert, S., H. Derradji, and S. Baatout, Telomere biology in mammalian germ cells and during development. Dev Biol, 2004. 274(1): p. 15-30.

101. Harley, C.B., Telomere loss: mitotic clock or genetic time bomb? Mutat Res, 1991. 256(2-6): p. 271-282.

102. Harley, C.B., et al., The telomere hypothesis of cellular aging. Exp Gerontol, 1992. 27(4): p. 375-382.

103. Baird, D.M. and D. Kipling, The extent and significance of telomere loss with age. Ann N Y Acad Sci, 2004. 1019: p. 265-268.

104. Liu, L., et al., Genetic and epigenetic modulation of telomerase activity in development and disease. Gene, 2004. 340(1): p. 1-10.

105. Boukamp, P., Ageing mechanisms: the role of telomere loss. Clin. Exp. Dermatol., 2001. 26(7): p. 562-565.

106. Ahmed, A. and T. Tollefsbol, Telomeres and telomerase: basic science implications for aging. J. Am. Geriatr. Soc., 2001. 49(8): p. 1105-1109.

107. Huffman, K.E., et al., Telomere shortening is proportional to the size of the G-rich telomeric 3'-overhang. J Biol Chem, 2000. 275(26): p. 19719-19722.

108. Kierszenbaum, A.L., Telomeres: more than chromosomal non-sticking ends. Mol Reprod Dev, 2000. 57(1): p. 2-3.

109. Nakagawa, S., N.J. Gemmell, and T. Burke, Measuring vertebrate telomeres: applications and limitations. Mol Ecol, 2004. 13(9): p. 2523-2533.

110. Prowse, K.R. and C.W. Greider, Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proc Natl Acad Sci U S A, 1995. 92(11): p. 4818-4822.

111. Campisi, J., Replicative senescence: an old lives' tale? Cell, 1996. 84(4): p. 497-500.

112. Goyns, M.H., Genes, telomeres and mammalian ageing. Mech Ageing Dev, 2002. 123(7): p. 791-799.

113. Lin, K.W. and J. Yan, The telomere length dynamic and methods of its assessment. J Cell Mol Med, 2005. 9(4): p. 977-989.

114. Gil, M.E. and T.L. Coetzer, Real-time quantitative PCR of telomere length. Mol Biotechnol, 2004. 27(2): p. 169-172.

115. ABI, Protocol: SYBR Green PCR Master Mix and RT-PCR. 2002.

116. ABI, Protocol: SYBR Green PCR and RT-PCR Reagents. 2001.

117. Higuchi, R., et al., Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology (N Y), 1993. 11(9): p. 1026-1030.

118. Boulay, J.L., et al., Gene dosage by quantitative real-time PCR. Biotechniques, 1999. 27(2): p. 228-230, 232.

119. Wang, M. and A. Marin, Characterization and prediction of alternative splice sites. Gene, 2006. 366(2): p. 219-227.

120. Goh, S.H., et al., A newly discovered human alpha-globin gene. Blood, 2005. 106(4): p. 1466-1472.

121. Goh, S.H., et al., Identification of an alternate delta-globin mRNA in adult human erythroid cells. Genomics, 2004. 84(2): p. 431-434.

122. Juusola, J. and J. Ballantyne, Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification. Forensic Sci. Int., 2003. 135(2): p. 85-96.

123. Kacharmina, J.E., P.B. Crino, and J. Eberwine, Preparation of cDNA from single cells and subcellular regions. Methods Enzymol., 1999. 303: p. 3-18.

124. Phillips, J. and J.H. Eberwine, Antisense RNA Amplification: A Linear Amplification Method for Analyzing the mRNA Population from Single Living Cells. Methods, 1996. 10(3): p. 283-288.

125. Sambrook, Molecular Cloning: a laboratory manual. 2001: CSHL Pres, Cold Spring Harbor.

126. Slightom, J.L., A.E. Blechl, and O. Smithies, Human fetal G gamma- and A gamma-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. Cell, 1980. 21(3): p. 627-638.

127. Proudfoot, N.J. and G.G. Brownlee, 3' non-coding region sequences in eukaryotic messenger RNA. Nature, 1976. 263(5574): p. 211-214.